TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE BASED ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS

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I hereby declare that the thesis entitled **"TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE BASED ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS"** submitted towards partial fulfillment of the requirements for the award of **MS GENERAL SURGERY** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Ashvind Bawa,** Professor, Department of General Surgery, **Dr. Jyoti Chaudhary**, Professor, Department of Microbiology. No part of this thesis has formed the basis for the award of any degree previously.

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Dedicated

to

My Lord &

Saviour

Jesus Christ

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ABBREVIATIONS

DFU – Diabetic Foot Ulcer

HAPI – Hospital Acquired Pressure Injuries

MRSA – Methicillin-Resistant Staphylococcus aureus

PCR – Polymerase Chain Reaction

rRNA – Ribosomal Ribonucleic Acid

NADP – Nicotinamide Adenine Dinucleotide Hydrogen

NADPH - Nicotinamide Adenine Dinucleotide Phosphate hydrogen

CFU – Colony Forming Units

ABSTRACT

INTRODUCTION: Illuminate®, a novel handheld device developed based on multi- spectral imaging of autofluorescence exhibited by bacterium has proven to be an early screening device in the identification and classification of bacteria. The non-invasive and less time-consuming procedure are the added advantages of the device. Routine microbial-based culture methods, the current standard for identifying and classifying bacteria infecting the wounds, requires taking a swab or tissue from the infected region and take 2 to 3 days for diagnosis

OBJECTIVE: To rapidly identify and classify gram positive and negative organisms in wounds using artificial intelligence device.

•To evaluate predictability and see efficacy of artificial intelligence device in gram positive or gram negative infections in wounds.

MATERIALS AND METHODS: The study is a prospective observational study carried out in the department of general surgery, Dayanand medical college and hospital, Ludhiana.

- Patient's wound imaging were taken subsequently using illuminate® and at the same time wound tissue was sent for culture. Illuminate® suggested gram positive and negative forms and was compared to culture report from the tissue.
- Sample size of 100 was taken and studied in one year duration using the artificial intelligence (AI) device

RESULTS: The overall accuracy of 72% suggests that while the Illuminate® device is a promising tool, but there is room for improvement. The high sensitivity (88.89%) for Gram-negative bacteria suggests that the device is effective at identifying these infections. However, the low specificity (35.71%) indicates a tendency for false positives, which could lead to over treatment if not interpreted cautiously. For Gram-positive bacteria, the device showed high specificity (98.90%) but low sensitivity (11.11%). This suggests that while the device may miss some Gram-positive infections, when it does indicate their presence, it is likely to be accurate. The device's performance in identifying cases with no bacterial growth was characterized by very high specificity (95.24%) but low sensitivity (25.00%). This implies that while the device rarely misidentifies uninfected wounds as infected, it may not reliably detect all uninfected wounds.

CONCLUSIONS: In conclusion, the Illuminate® device demonstrates potential as a rapid, non-invasive tool for bacterial detection in wound ulcers. Its high sensitivity for Gram-negative bacteria and high specificity for Gram-positive bacteria make it a valuable adjunct to clinical assessment. However, its limitations, particularly in terms of specificity for gram-negative bacteria and sensitivity for uninfected wounds, should be considered in its clinical application.

KEY WORDS: Autofluorescence, Illuminate®, Gram-positive & Gram-negative bacteria, Wound, Infections.

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INTRODUCTION

Wounds are a result of the interruption of the anatomical and physiological integrity of the skin. When discussing the process of healing, wounds can be categorised as either acute or chronic. A wound is classified as chronic if it fails to heal within the anticipated timeframe based on its cause and location. Chronic ulcers are divided into two main types: typical and atypical. Common types of wounds include ischemic, neurotrophic, and hypostatic ulcers, as well as two most common conditions: diabetic foot ulcers and decubitus ulcers. Approximately 80% of chronic wounds found on the lower leg are caused by chronic venous insufficiency, 5-10% are caused by arterial problems, and the rest are mostly neuropathic ulcers [1].

Infections are the primary cause of illness and death related to foot ulcers, occurring in 40-80% of cases [2]. When subcutaneous tissue is exposed, it creates a suitable habitat for a wide range of microorganisms to contaminate and colonise, resulting in tissue damage. When the host's immune system is weakened, it creates ideal conditions for the proliferation of microorganisms [3].

At present, the conventional approaches for identifying and categorising bacteria causing wound infections involve the use of normal microbial-based culture techniques. This procedure necessitates obtaining a swab sample or biopsy taken from the affected area and usually takes 48 to 72 hours for the determination of the diagnosis. The delay in administering initial treatment and/or the possible misuse of antibiotics can result in the emergence of bacterial resistance [4].

Recent breakthroughs in medical technology have prompted the investigation of innovative diagnostic techniques. An effective strategy utilises the concept of bacterial autofluorescence. Bacteria possess varied quantities of fluorophores within distinct micro-environments, facilitating the differentiation of different bacterial species [5].

Expanding upon this premise, a new portable device called Illuminate® has been created. This device uses multi-spectral imaging to detect the

autofluorescence emitted by bacteria. It has demonstrated potential as an early screening tool for identifying and categorising bacteria in wounds. The gadget evaluates the metabolic and infectious signs of the underlying pathogens, showing high overall precision while excluding the identification of harmless microorganisms. This treatment is non-invasive and quick, which provides notable benefits compared to conventional methods [6].

A comprehensive understanding of the interplay in the inflammation and microorganisms is important in the diagnosis of skin ulcer infections in contemporary wound care. The presence of bacteria in ulcers often progresses from contamination to colonisation, then to critical colonisation, and finally to infection [7].

The precise mechanism by which colonisation affects wound healing is not well comprehended, however it is primarily linked to aerobic or facultative microorganisms, such as Pseudomonas aeruginosa, Staphylococcus aureus, and beta-hemolytic streptococci. The suggested association between anaerobic species and delayed wound healing may be more attributable to the unavailability of anaerobic culture techniques in research investigations, rather than a lack of pathogenicity. Although anaerobic organisms pose challenges in terms of culturing and identification, they have been discovered to account for 30% of all microbial isolates in wounds [8-10].

The bacterial colonisation in wounds undergoes temporal variations. In wounds that are less than one month old, the most common types of organisms present are those that affect the skin and are classified as Gram-positive. As the process of healing of an ulcer advances, a wider variety of organisms, including Gram-negative organisms such as anaerobes, coliforms, and Pseudomonas species, may inhabit the wound [11].

Within this particular framework, the utilisation of artificial intelligence devices such as Illuminate® should be regarded as an innovative approach for the identification of wound infections. These gadgets can offer crucial information about wounds that do not improve or demonstrate signs of severe colonisation. Although culture methods

can provide information on the prevalent microorganisms in wounds, identify resistant species, and help determine the appropriate systemic treatment for infected wounds, these emerging technologies have the potential to provide quick and convenient diagnostic tests at the point of care.

This study, carried out at DMCH Ludhiana, seeks to assess the effectiveness of the Illuminate® instrument in identifying the specific types of microorganisms present in wound ulcers. The findings of this study could assist in the early implementation of antibiotic treatment and enhancing overall strategies for wound management. Through the examination of the capabilities of this innovative technology, our goal is to make a valuable contribution to the progress of diagnosing wound care and ultimately enhance the results for patients in the complex area of managing chronic wounds.

AIMS AND OBJECTIVES

- To rapidly identify and classify gram positive and negative organisms in wounds using artificial intelligence device.
- To evaluate predictability and see efficacy of artificial intelligence device in gram positive or gram negative infections in wounds.

REVIEW OF LITERATURE

The history of wound healing is essentially synonymous with the history of humanity. An ancient medical book, a clay tablet from 2200 BC, provides an early description of the "three healing gestures" which include cleansing the wounds, preparing the plasters, and applying bandages [12-14].

Process of Wound Healing

Wound healing is an intricate and permanent process that involves three primary stages: inflammation, tissue formation, and tissue remodelling. This complex process entails a series of cellular and molecular activities that collaborate harmoniously to repair the integrity of the tissue [1].

1. The inflammation phase commences promptly following the injury and endures for approximately 2-5 days. It encompasses the processes of homeostasis and inflammation. Platelets gather at the site of injury, coming together to create a fibrin clot that halts bleeding and serves as a temporary framework for cell movement. Neutrophils and macrophages migrate into the wound site, eliminating waste material and microorganisms [2].

2. Proliferation Phase: This phase usually takes place between the second and twenty-first day after the injury. It is defined by the development of granulation tissue, the process of re-epithelialisation, and the production of new blood vessels. Fibroblasts travel towards the wound, multiply, and generate fresh extracellular matrix. Endothelial cells are responsible for the formation of new blood vessels, while epithelial cells move across the surface of the wound [3].

3. Remodelling Stage: This stage may last for a duration of one year or longer. During this period, the recently developed tissue undergoes reorganisation to closely resemble the original tissue. Collagen undergoes remodelling and realignment, and any cells that are no longer necessary are eliminated through apoptosis [4].

If the regular healing process is interrupted, an ulcer can become chronic due to a deficiency of growth factors and cytokines, which

cause a delay in the healing process [5]. Chronic wounds are distinguished by a longer phase of inflammation, prolonged infections, drug-resistant microbial biofilms, and inability to heal [6].

Wound Classification

Wounds can be categorised as either acute or chronic based on their healing process. A wound is classified as chronic if it fails to heal within the expected timeframe based on its cause and location. Chronic wounds are classified into two categories: typical and atypical [12]. Common examples of chronic wounds include:

1. Ischemic ulcers
2. Neurotrophic ulcers
3. Hypostatic ulcers
4. Diabetic ulcers
5. Decubitus ulcers, often known as pressure ulcers

Atypical wounds are wounds that do not fit into the standard classifications and can be caused by uncommon disorders including autoimmune diseases, infections, or cancers [13]. The majority (80%) of chronic wounds on the lower leg are attributed to chronic venous insufficiency, whereas 5-10% are caused by arterial issues. The remaining wounds are mainly neuropathic ulcers [14].

Diabetic Foot Ulcers (DFU)

Diabetic foot ulcers are a notable consequence of diabetes mellitus, constituting one of the most difficult and expensive problems to manage. DFUs refer to deep wounds that occur in pressure-bearing areas or areas exposed to trauma below the ankle in patients with diabetes [7]. The pathophysiology of diabetic foot ulcers (DFU) lies in the underlying biological processes that progresses to the development and progression of these ulcers in individuals with diabetes.

The etiology of DFU is characterized by a three of components [8]:

1. Neuropathy: Sensory and motor neuropathy both have significant functions. Sensory neuropathy results in the loss of the ability to feel sensations, which makes the foot susceptible to undiscovered injuries. Motor neuropathy results in muscle wasting and foot abnormalities, which in turn cause the formation of aberrant pressure points [9].

2. Vascular insufficiency: A condition where there is inadequate blood flow to the extremities. In the case of diabetes, it is linked to the hastened development of atherosclerosis, which further contributes to the reduced blood flow. This hinders the process of healing and enhances vulnerability to infection [10].

3. Immunopathy: Diabetes hampers immunological function, heightening vulnerability to infection and hindering the wound healing process [11].

Wagner's categorisation of diabetic foot ulcers: Wagner's classification system categorises the extent of diabetic ulcers on a scale ranging from 0 to 5 [15]:

Grade 0: Absence of open lesions; potential presence of deformity or cellulitis

Grade 1: Superficial diabetic ulcer (involving either partial or whole thickness of the skin)

Grade 2: Ulcer that has spread to the ligament, tendon, joint capsule, or deep fascia, without the presence of an abscess or osteomyelitis.

Grade 3: Severe ulcer with an abscess, infection of the bone (osteomyelitis), or infection of the joint (joint sepsis)

Grade 4: Gangrene is confined to a specific area of the forefoot or heel.

Grade 5: Severe and widespread tissue death in the foot due to gangrene

This classification system aids in selecting the suitable treatment approach and prognosis for every patient.

Pressure ulcers

Also called decubitus ulcers, are skin interruption that occur due to prolonged pressure at dependent areas of the body. Pressure ulcers are specific injuries that occur on the dermis or underlying tissue, typically over a bony area, due to sustained pressure or a combination of pressure with shear or friction [16]. The occurrence of pressure injuries is intricate and multifaceted, encompassing both external and internal variables.

External influences comprises the following [17]:

1. Extended application of force: When soft tissue is squeezed between a protruding bone and an external surface for a long time, it can result in reduced blood flow, insufficient oxygen supply, and ultimately death of the tissue.
2. Shear forces refer to the occurrence of skin layers sliding over each other, which leads to the disruption of blood circulation in that area.
3. Friction: This can result in the abrasion of outer layers of skin, making it more susceptible to pressure injury.
4. Excessive moisture, typically caused by incontinence or perspiration, can cause the skin to become macerated, hence increasing its vulnerability to harm.

Internal factors encompass a variety of elements, which can be categorised into distinct components [18].

1. Malnutrition hampers the healing of wounds and reduces the ability of tissues to withstand pressure.
2. Anemia: Decreases the transportation of oxygen to tissues, which hinders the process of healing.
3. Endothelial dysfunction, which is prevalent in illnesses such as diabetes, hampers the flow of blood through small blood vessels and the delivery of blood to tissues.

The predominant risk factors for pressure ulcers are Reduced mobility, Malnutrition, Urinary or fecal incontinence, Cognitive impairment, Elderly, Coexisting medical diseases that impact the process of tissue healing, such as diabetes and peripheral vascular disease [19].

Braden Scale for Pressure Sore Risk Prediction:

The Braden Scale is a commonly employed scale for evaluating a patient's susceptibility to getting pressure ulcers. The measurement consists of six sub-scales [20] i.e. Sensory perception, humidity, activity, mobility, nutrition, friction

The rating scale for each sub-scale ranges from 1 to 4, except for Friction and Shear which ranges from 1 to 3. Lower scores on the scale indicate a higher level of danger. The overall score spans from 6 to 23, with scores of 18 or below suggesting a state of being at danger.

Study of the occurrence and distribution of long-lasting wounds

Chronic wounds provide a substantial worldwide health challenge. Their occurrence is on the rise as a result of aging populations and growing rates of obesity, diabetes, and vascular disease [21].

Diabetic foot ulcers(DFU):

Diabetes mellitus impacts over 422 million individuals globally and is accountable for an approximated 2 million fatalities annually [22]. The United States population is affected by it at a rate of 11.3% [23]. Around 15% of people with diabetes may eventually get a foot ulcer, and out of these individuals, between 14% to 24% will need to undergo amputation as a result of complications associated to the ulcer [24].

Decubitus ulcers:

Pressure ulcers have a global impact, affecting thousands of individuals annually [25]. Annually, around 3 million adults in the United States are impacted [26]. Pressure injury management poses a substantial financial burden. According to a study conducted by Padula and Delarmente in 2019, the yearly expenses of hospital-acquired pressure injuries (HAPI) in the United States could surpass $26.8 billion [27]. The prevalence of pressure ulcers varies based on the healthcare environment. The range of acute care is from 0.4% to 38%.The range of long-term care varies from 2.2% to 23.9%. Home care: ranging from 0% to 17%. Specific patient populations that are more susceptible to risk include [28] Approximately two-thirds of ulcers develop in patients who are over the age of 70, making them predominantly prevalent among elderly individuals. Patients with a severe medical condition like neurological impairments, such as individuals with spinal cord injuries, individuals undergoing extended surgical procedures

Chronic wounds histopathology

Gaining a comprehensive understanding of the histopathology of chronic wounds is essential for obtaining valuable insights into the obstacles that hinder the healing process and for directing therapeutic approaches.

Diabetic foot ulcers:

DFUs can be identified through histopathological investigation, which displays distinct traits [29]:

1. Epidermal Changes: It is common to see areas of thickened epidermis and excessive keratin production, along with damage to the outer layer of skin.

2. Dermal Changes: The dermis has a persistent inflammatory process, predominantly consisting of circular mononuclear cells. Regions with significant collagen fibrosis are frequently seen, and on occasion, calcium salt deposits can be seen.

3. Vascular involvement: The majority of arteries and arterioles display arteriosclerotic modifications, which contribute to the ischemic characteristic of these ulcers.

4. The immunohistochemistry studies reveal an abundance of CD4+ T-lymphocytes, with a smaller number of CD3+ T-lymphocytes, indicating an inflammatory infiltrate. This ongoing inflammatory condition contributes to the enduring characteristic of these wounds.

Decubitus ulcers:

The histology of pressure ulcers demonstrates a progressive process that advances through multiple stages [30]:

1. In the early stage, first alterations can be detected in the blood vessels located in the papillary dermis. Blanchable and non-blanchable erythema are frequently observed.

2. Progressive Stage: As the ulcer advances, there is observable decubitus dermatitis, which is marked by an inflammatory infiltration and initial tissue degradation.

3. Advanced Stage: In pressure ulcers that have reached complete development, there is widespread necrosis of the skin structures. The ulcer has the ability to penetrate deeper tissues, maybe reaching the muscle or even the bone.

4. In chronic stage pressure ulcers, histopathology commonly shows the existence of tightly clustered bacterial colonies inside the extracellular matrix. This observation is generally absent in ulcers that form rapidly and contributes to the long-lasting nature of the lesion.

Studies examining the histology of exposed bone in sacral pressure sores have revealed a low occurrence of osteomyelitis. If the infection is present, it often affects a specific area and is limited to the outer layers of the cortical bone [31].

The microbiology of chronic wounds is intricate and has a vital impact on the process of wound healing. Gaining knowledge about the microbial ecology of these wounds is crucial for ensuring effective treatment.

Continuum of Bacterial Colonisation:

Bacteria in wounds generally progress through a series of stages, starting with contamination, then colonisation, followed by critical colonisation, and ultimately leading to infection [32]. This sequence can be characterised as follows:

1. Contamination refers to the existence of non-replicating bacteria in the wound.
2. Colonisation refers to the presence of multiplying bacteria that are attached to the surface of a wound, but do not cause any harm to the surrounding tissue.
3. Critical colonisation refers to the rapid multiplication of organisms and the initial onset of tissue damage in a specific location, without a noticeable response from the host organism.
4. Infection refers to the process in which microorganisms invade and multiply in wound tissue, resulting in harm to the tissue and triggering a response from the host.

Typical Disease-Causing Microorganisms:

The predominant microorganisms implicated in chronic wound infections include [33] Staphylococcus aureus, which includes Methicillin-Resistant S. aureus or MRSA, Pseudomonas aeruginosa, Beta-hemolytic streptococci, Enterococci, Escherichia coli, Klebsiella species, Proteus species. Examples of anaerobic bacteria are Bacteroides and Peptostreptococcus. The bacterial colonisation in chronic wounds undergoes temporal shifts [34]. In the first stage (< 1 month), the infection is mainly limited to the skin and caused by Gram-positive microorganisms. In the later stages, there is an increased

variety of organisms, including Gram-negative bacteria such Pseudomonas species, coliforms, and anaerobes.

Biofilms are highly influential in the long-lasting presence of chronic wounds. These are biofilms, which consist of microorganisms that bind to surfaces and are surrounded by a self-secreted extracellular polymeric material. Biofilms exhibit a well-known ability to withstand antimicrobial treatments and host immunological responses, which leads to the prolonged existence of wounds [35].

Prompt and precise identification of wound infections is essential for efficient treatment. Conventional and new diagnostic techniques include distinct advantages and drawbacks. Clinical examination is the initial step in diagnosing a wound infection. Indicators and manifestations of infection encompass the following [36] Erythema refers to redness of the skin caused by dilation of blood vessels. Rise in temperature, inflammation, pain, pus discharge, foul odour, Prolonged wound healing. Nevertheless, these indications may be inconspicuous in long-lasting injuries, especially in individuals with weakened immune systems or neuropathy.

Swab cultures are a conventional technique used to detect and identify microorganisms in wounds. The Levine technique, which involves applying pressure to a 1 cm^2 area of the wound with a rotating swab to remove fluid from the wound tissue, is frequently employed [37]. Nevertheless, swab cultures possess certain limitations. They may not provide an accurate representation of the complete wound microbiota. They may fail to detect profound tissue infections. They may acquire superficial pollutants that are not responsible for the infection.

Biopsy of tissue:

Tissue biopsy is often regarded as the most reliable method for identifying wound infections. It offers data on the existence of microbes and their infiltration into living tissue. Nevertheless, it is intrusive and may not be feasible for regular surveillance [38].

Methods based on the study and manipulation of molecules:

The Polymerase Chain Reaction (PCR) and 16S rRNA sequencing techniques offer quick and comprehensive insights into the composition

of the wound microbiome. These techniques have the ability to identify species that are challenging to grow in a lab and offer a more complete understanding of the microbial ecosystem. Nevertheless, they come at a higher cost and have the ability to identify non-viable organisms [39].

Advancing Technologies:

Emerging technologies are being created to offer swift and on-site detection of wound infections. One example of a technology is founded on the notion of bacterial autofluorescence.

Autofluorescence imaging:

Autofluorescence is the phenomenon where biological structures emit light after absorbing light, and this is generated by naturally occurring fluorophores. Within the context of wound infections, some bacterial components display autofluorescence [40]. NADH and NADPH coenzymes emit fluorescence in the form of blue/green region of the electromagnetic spectrum. Flavins also cause the emission of blue and green light. Porphyrins are responsible for the production of red fluorescence. Pyoverdine is a siderophore that is synthesised by Pseudomonas aeruginosa and has a distinct blue fluorescence.

The Illuminate® Device:

Fig 1: Illuminate® device

The Illuminate® gadget, developed by Adiuvo Diagnostics in Chennai, India, is an innovative handheld device that utilizes the natural fluorescence of bacteria to quickly detect infections. (Fig 1. Illuminate® device)The device's main characteristics consist of that it produces light with wavelengths of 370, 390, and 415 nm. It acquires a sequence of 16 multispectral images within a time frame of 30 seconds. The test yields outcomes indicating the presence of gram-positive, gram-negative, or no infection within a short span of time. The snapshot shows the results, with gram-negative bacteria highlighted in green and gram-positive bacteria highlighted in red.

Benefits of Autofluorescence Imaging involves Quick outcomes (within minutes), noninvasive , more accurate wound sampling, Ability to direct precise removal of dead tissue, Capability to continuously monitor the level of germs present over a period of time without the need for repetitive invasive sampling.Can identify germs in the periwound region that can go unnoticed during a regular examination [6].

Disadvantages of Autofluorescence Imaging:

- Lacks the ability to differentiate between individual bacterial species
- May be influenced by other fluorescent compounds present in the wound, such as certain topical therapies.
- Requires a dimly lit environment for optimal imaging
- The depth to which it can reach is limited, and it mostly evaluates bacteria on the surface and in the immediate vicinity.

Research on Autofluorescence Imaging:

1. Viswanathan V et al 8(2021) examined the device Illuminate®. The study aimed to assess the diagnostic accuracy of a device in identifying bacterial Gram types compared to standard culture methods. Researchers recruited 178 patients from a diabetes tertiary care center and collected 203 tissue samples from the wound base, as performed by the surgeon. The results were compared with the Gram type interpretation provided by the device. The device demonstrated an overall accuracy of 89.54%, with a positive predictive value of 86.27% for detecting Gram-positive bacteria, 80.77% for Gram-negative

bacteria, and 91.67% for identifying no infection. The negative predictive value was 87.25% for Gram-positive bacteria, 92% for Gram-negative bacteria, and 96.12% for no infection [6].

2. Koenig et al. (1994) provided a detailed account of using fluorescence to identify a range of biomolecules and illnesses, including wound infections. The authors emphasized the capability of autofluorescence in identifying initial-stage wound infections, dental caries, and other medical problems [31].

3. Wu et al. (2014) created a first portable autofluorescence imaging device named PRODIGI to directly observe the amount of bacteria in wounds in real-time. In their preclinical experiments using a mouse model, they were able to detect [32] Staphylococcus aureus in wounds that were not visible during normal examination.

4. Chamma et al. (2017) employed autofluorescence image-guided sampling using MolecuLight to detect medically important bacteria in diabetic foot ulcers. Implementing this method enhanced the precision of collecting samples from wounds for the purpose of microbiological analysis [33].

5. DaCosta et al. (2015) conducted a trial to evaluate the practicality of using autofluorescence imaging to guide the collection of samples from chronic wounds. The researchers discovered that the presence of bacteria may be rapidly detected and accurately measured over a period of time [34].

MATERIALS AND METHODS

SOURCE OF DATA

Data was collected from all patients with wounds who presented to DMCH in the time period of October 2022 to September 2023 according to the inclusion and exclusion criteria

METHOD OF COLLECTION OF DATA

Information was collected through a predesigned pre-tested proforma for each patient.

- All patients were interviewed according to the proforma, and a thorough clinical examination was conducted.
- Patient's wound imaging were taken using ILLUMINATE®
- Cultures of patients were collected either pre or post operatively in the form of tissue culture at the same time of imaging.Culture samples were sent in sterile containers.

Inclusion criteria

- Adult patients (>18 years) with acute and chronic wounds, including diabetic ulcers, arterial ulcers, post-op ulcers, traumatic ulcers, pressure ulcers, and venous ulcers were taken
- Both Acute and chronic wounds were taken
- Both inpatient and outpatients were taken

Exclusion criteria

- Age less than 18 years
- Underlying bone involvement like osteomyelitis
- Patients with infections under intact skin or pre-existing skin conditions like eczema and psoriasis were excluded.
- Patients not giving consent for the study

Sample size: Time bound study of 1 year with total of 100 patients.

Sample size calculation: The reference study used for calculation of sample size was by Vijay Viswanathan et al (2021) in which authors reported that the accuracy of device was found to be 89.54% on comparing with the gold standard.

Cochran formula used for calculation of Sample size is as follows

$n = (Z^2\alpha \text{ X } P \text{ X } (1\text{-}P))/d^2$

Where

Z α is the level of significance at 5% i.e. 95% confidence interval = 1.96

P = Proportion of accuracy is 89.54% = 0.8954, as reported in the reference study used.

d = Desired error of margin = 6% = 0.06
Hence 100 patients are needed in the study.

Duration of the study: October 2022 to September 2023
Outcome: Knowing the efficacy of the artificial intelligence device in differentiating gram positive or negative infection or no infection in wound ulcer.

STATISTICAL ANALYSIS:

Data were described in terms of range; mean ±standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. For calculating agreement between the methods kappa value was calculated. Sensitivity, specificity, positive and negative predictive values and accuracy of each method and its confidence interval (95% CI) were also determined. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using (Statistical Package for the Social Science) SPSS 21.0 version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

OBSERVATIONS AND RESULTS

This prospective study involved 100 patients with wound ulcers of various aetiologies presenting to our hospital from October 2022 to September 2023. Patient's wounds were examined and images were taken using ILLUMINATE®. Subsequently, tissue cultures were taken from the targeted site and comparison was done.

Demographics:

- 100 patients were included in the study
- Age distribution: 53% were between 51-70 years old

Table 1: Age distribution of the patients taken in the study

Age group	No. of cases	Percentage
< 30	9	9.0%
31-40	6	6.0%
41-50	15	15.0%
51-60	31	31.0%
61-70	22	22.0%
> 70	17	17.0%
Total	100	100.0%

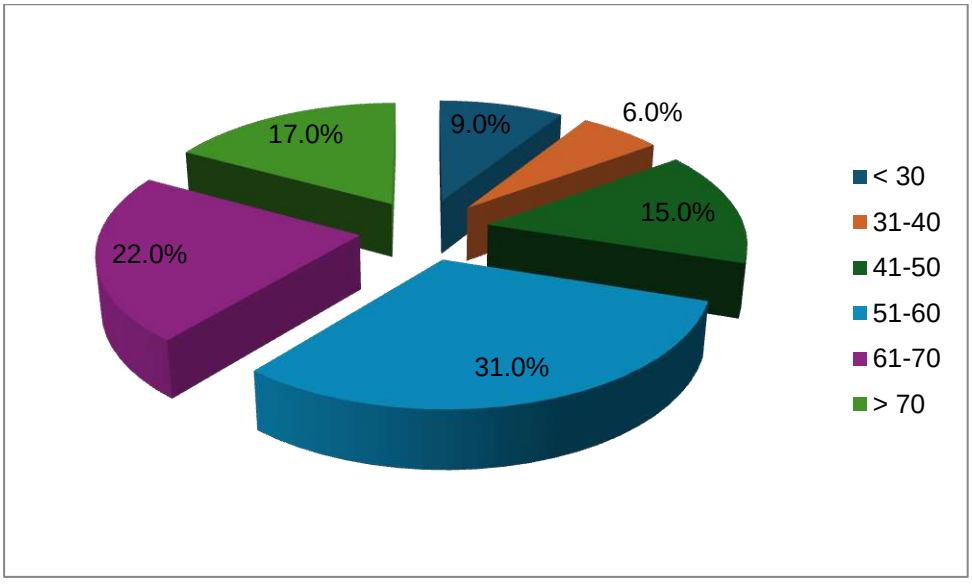

Fig 2: Pie diagram showing Age distribution

Table 2: Gender distribution

- Gender distribution: 77% male, 23% female

Gender	No. of cases	Percentage
F	23	23.0%
M	77	77.0%
Total	100	100.0%

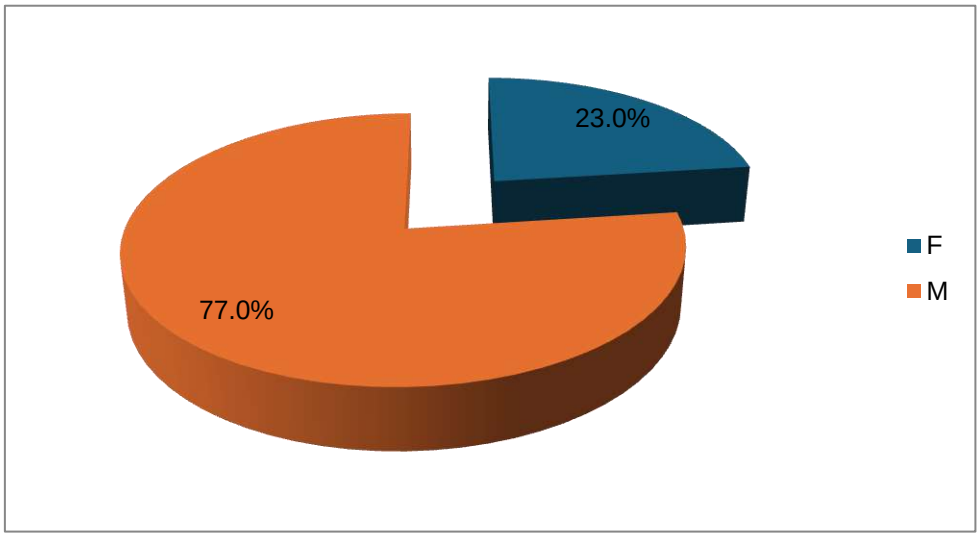

Fig 3: Pie chart showing Gender distribution

Table 3: Location of the Wounds

Wound Characteristics:

- Location: 45% on foot, 20% on leg, and rest were at other locations

Wound Location	No. of cases	Percentage
Abdominal	13	13.0%
Back	2	2.0%
Chest	1	1.0%
Foot	45	45.0%
Gluteal Region	6	6.0%
Hand	2	2.0%
Leg	20	20.0%
Perianal Region	4	4.0%
Pilonidal Region	1	1.0%
Sacrum	2	2.0%
Thigh	4	4.0%
Total	100	100.0%

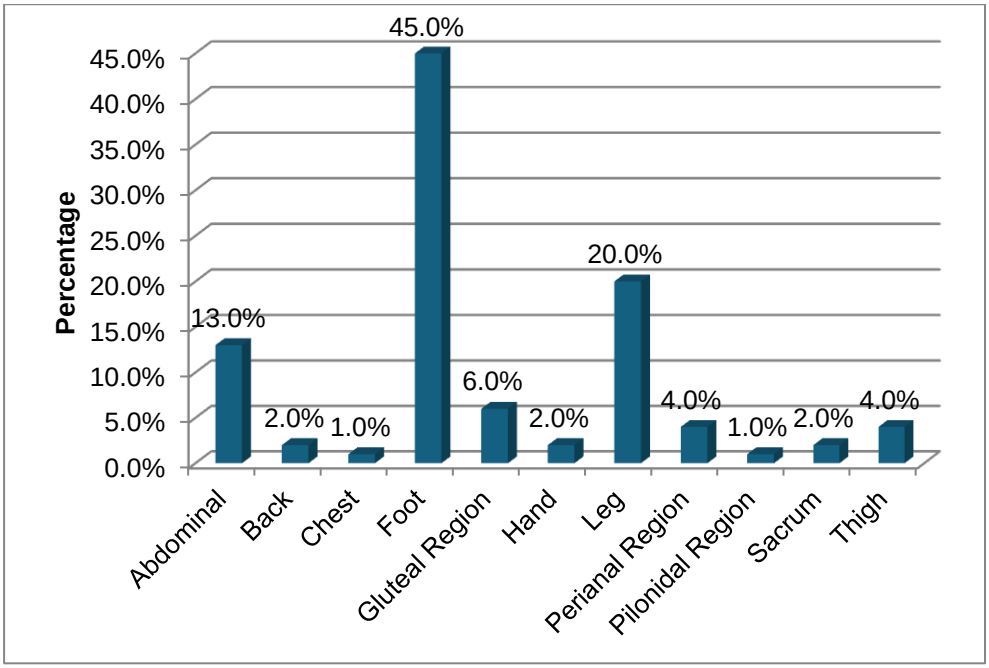

Fig 4: showing location distribution of the wounds taken in the study

Table 4: Etiology of the wounds taken

Etiology: 55% diabetic ulcers, 13% surgical site infections, 10% venous ulcers

Wound Aetiology	No. of cases	Percentage
Cellulitis	4	4.0%
Diabetic	55	55.0%
Fournier	1	1.0%
Maligant	1	1.0%
Non Healing Ulcer	1	1.0%
Others	1	1.0%
Pilonidal Sinus	1	1.0%
Pressure Sore	8	8.0%
SSIs	13	13.0%
Traumatic	4	4.0%
Venous And Diabetic	1	1.0%
Venous Ulcer	10	10.0%
Total	100	100.0%

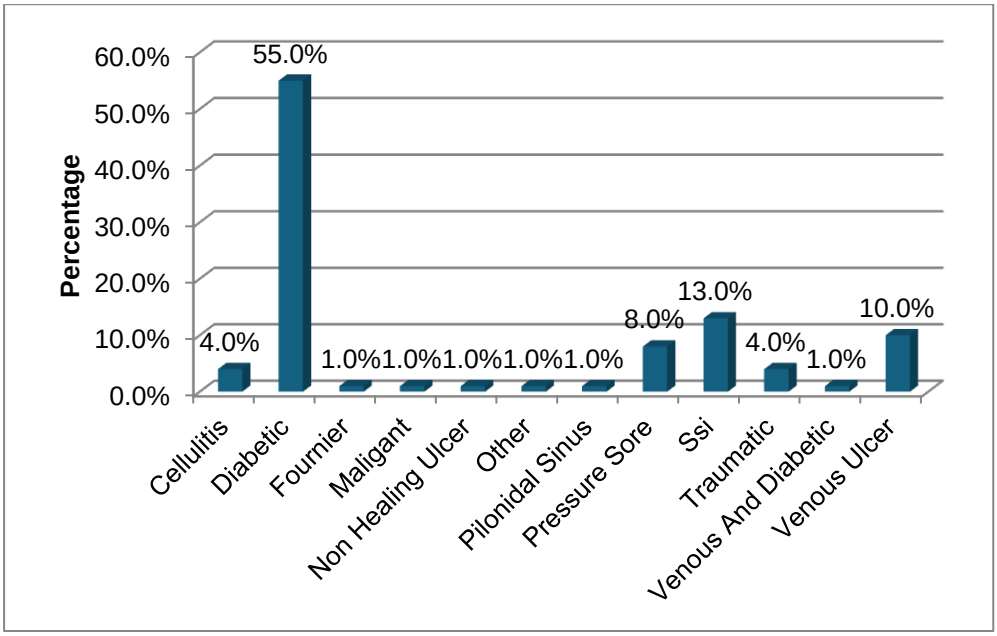

Fig 5: Showing various etiologies of the wound ulcers

Table 5: showing Exudate type in wound ulcers seen

- Exudate: 73% had no exudate, 25% had purulent exudate

Exudate Type	No. of cases	Percentage
None	73	73.0%
Purulent	25	25.0%
Serous	2	2.0%
Total	100	100.0%

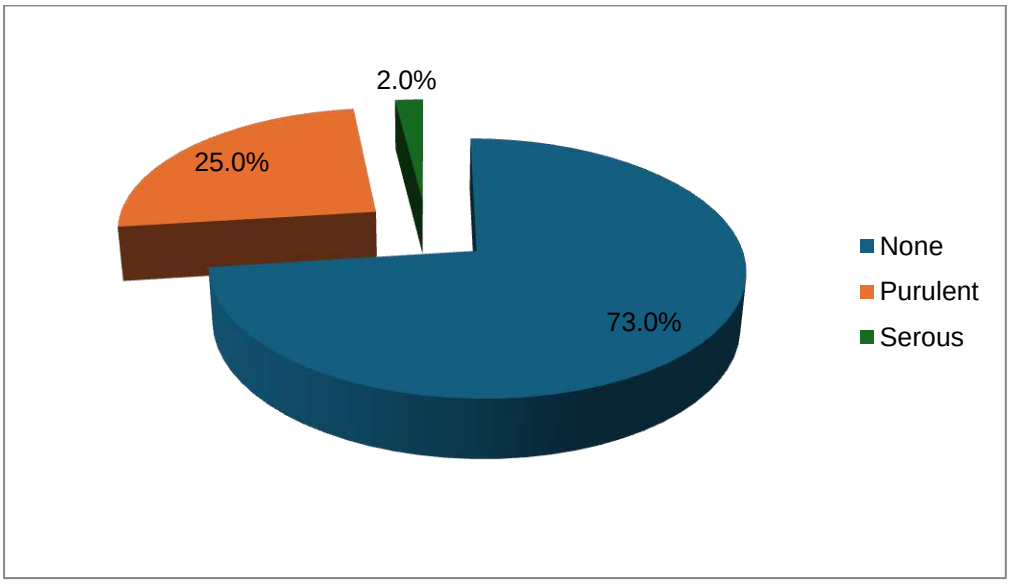

Fig 6: Pie chart showing distribution of type of exudate

Illuminate® Device Results:

- 82% showed Gram-negative bacteria
- 8% showed both Gram-negative and Gram-positive bacteria
- 2% showed Gram-positive bacteria only
- 8% showed no growth

Table 6: Device showing results of Wound image captured

Gram Negative	82	82.0%
Gram Negative and Gram Positive	8	8.0%
Gram Positive	2	2.0%
No Growth	8	8.0%
Total	100	100.0%

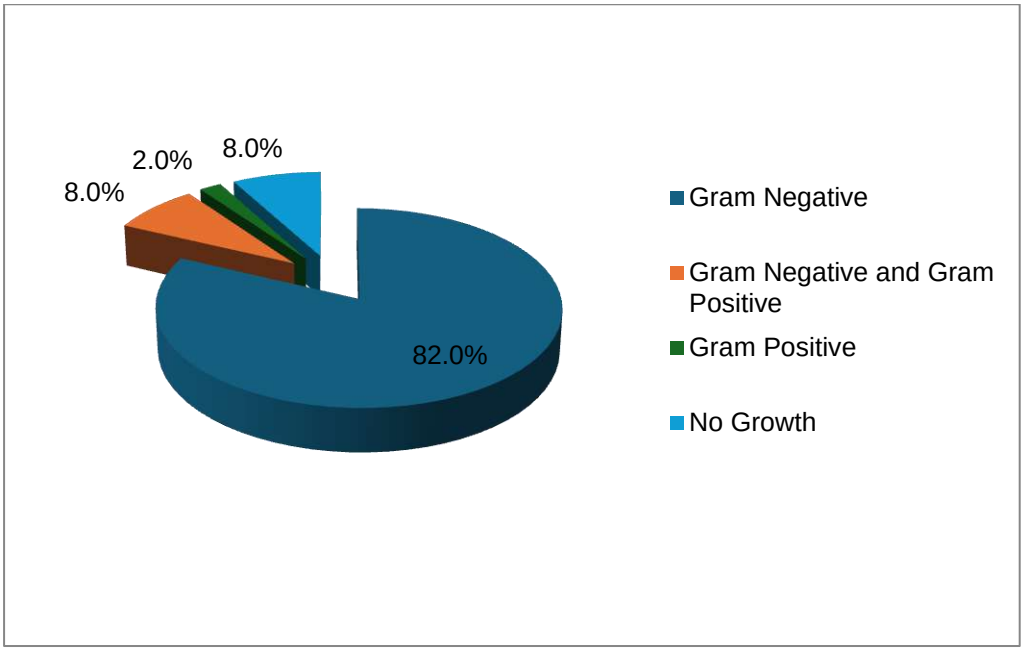

Fig 7: Results by the device showing Gram positives and negatives

Tissue Culture Results:

- 72% were positive for Gram-negative bacteria
- 8% showed both Gram-negative and Gram-positive bacteria
- 3% were positive for Gram-positive bacteria
- 16% showed no growth

Table 7: Results of the Tissue culture obtained from the wounds

Tissue Result	No. of cases	Percentage
Gram Negative	72	72.0%
Gram Negative and Gram Positive	3	3.0%
Gram Positive	9	9.0%
No Growth	16	16.0%
Total	100	100.0%

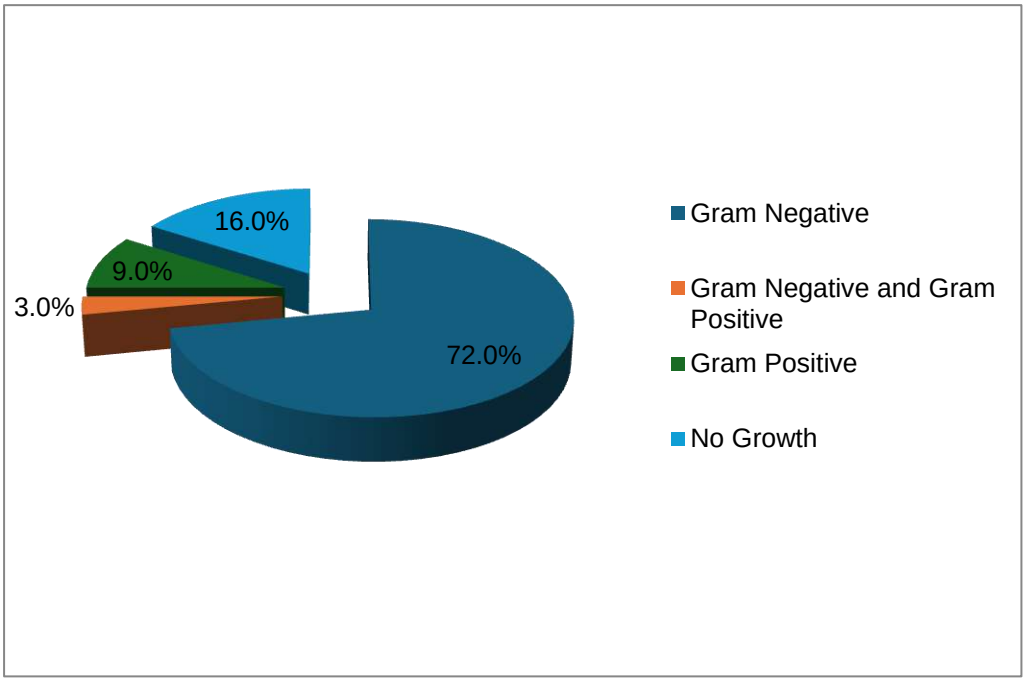

Fig 8: Results obtained from the tissue culture

Table 8: Bacteriology obtained from the wound ulcers

Organisms isolated:

Tissue Sample Result	No. of cases	Percentage
Acinetobacter baumannii	7	7.0%
Burkholderia cepacia	1	1.0%
Citrobacter koseri ,Enterococcus faecalis	1	1.0%
Diptheroids	1	1.0%
Enterobacter cloacae	2	2.0%
Enterococcus faecalis, Escherichia coli	2	2.0%
Enterococcus feacium	1	1.0%
Escherichia coli	22	22.0%
Escherichia coli, Pseudomonas aeruginosa	1	1.0%
Escherichia coli, Serratia marcescens	1	1.0%
Klebsiella Oxytoca	1	1.0%
Klebsiella pneumoniae	8	8.0%
Klebsiella pneumoniae, Acinetobacter baumannii	1	1.0%
Klebsiella pneumoniae, Enterobacter aerogenes	1	1.0%
Klebsiella pneumoniae, Pseudomonas aeruginosa	1	1.0%
No growth	16	16.0%
Proteus mirabilis, Escherichia coli	1	1.0%
Proteus vulgaris	1	1.0%
Pseudomonas aeroginosa, Providencia stuartii	1	1.0%
Pseudomonas aeruginosa	19	19.0%
Pseudomonas aeruginosa, Acinetobacter baumannii	1	1.0%
Pseudomonas aeruginosa, Escherichia coli	2	2.0%
Staphylococcus aureus	3	3.0%
Staphylococcus haemolyticus	2	2.0%
Staphylococcus hominis	1	1.0%
Staphylococcus Lentus, Escherichia coli	1	1.0%
Stenotrophomonas maltophilia	1	1.0%
Total	100	100.0%

Overall bacteriology suggests Gram Negative including Escherichia coli in 30% of wounds, Pseudomonas aeruginosa in 25%, Klebsiella pneumoniae in 12% and others in wounds.

Device Performance

Table 9: Showing combined results of the AI device and tissue cultures

		Tissue Result					Kappa value	p-value
		Gram Negative	Gram Negative and Gram Positive	Gram Positive	No growth	Total		
Illuminate Result	Gram Negative	64	0	6	12	82	0.287	0.001
	Gram Negative and Gram Positive	3	3	2	0	8		
	Gram Positive	1	0	1	0	2		
	No Growth	4	0	0	4	8		
Total	72		3	9	16	100		

Table 10: Results for Gram negative obtained on AI device

- For Gram-negative bacteria:

	Gram Negative	
Statistic	Value	95% CI
Sensitivity	88.89%	79.28% to 95.08%
Specificity	35.71%	18.64% to 55.93%
Positive Likelihood Ratio	1.38	1.04 to 1.84
Negative Likelihood Ratio	0.31	0.14 to 0.71
Disease prevalence	72.00%	62.13% to 80.52%
Positive Predictive Value	78.05%	72.72% to 82.58%
Negative Predictive Value	55.56%	35.49% to 73.96%
Accuracy	74.00%	64.27% to 82.26%

Sensitivity of gram negative is 88.9% (confidence interval - 79.28-95.08%) as compared to tissue culture report and specificity is 35.71 % (confidence interval - 18.64-55.93%). The overall accuracy of gram negative is 74% (confidence interval- 64.27-82.26%).

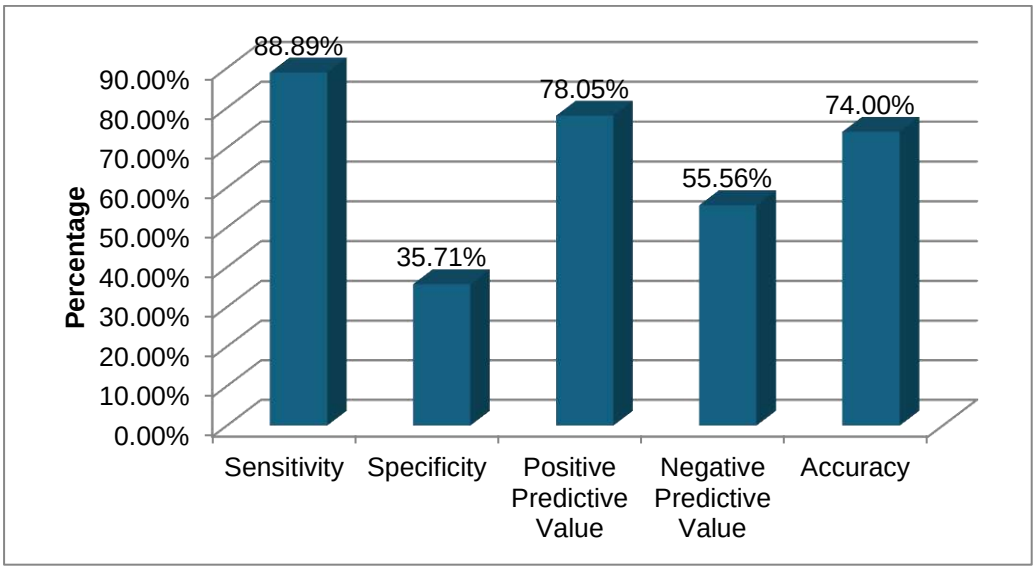

Fig 9: Results for Gram negative obtained on AI device

Table 11: Results for Gram positive obtained on AI device

For Gram-positive bacteria:

	Gram Positive	
Statistic	Value	95% CI
Sensitivity	11.11%	0.28% to 48.25%
Specificity	98.90%	94.03% to 99.97%
Positive Likelihood Ratio	10.11	0.69 to 148.35
Negative Likelihood Ratio	0.9	0.71 to 1.13
Disease prevalence	9.00%	4.20% to 16.40%
Positive Predictive Value	50.00%	6.38% to 93.62%
Negative Predictive Value	91.84%	89.92% to 93.42%
Accuracy	91.00%	83.60% to 95.80%

Sensitivity of gram positive is 11.11% (confidence interval- 0.28%-48.25%) as compared to tissue culture report and specificity is 98.90 % (confidence interval – 94.03-99.97%). The overall accuracy of gram positive is 91% (confidence interval- 83.60-95.80%).

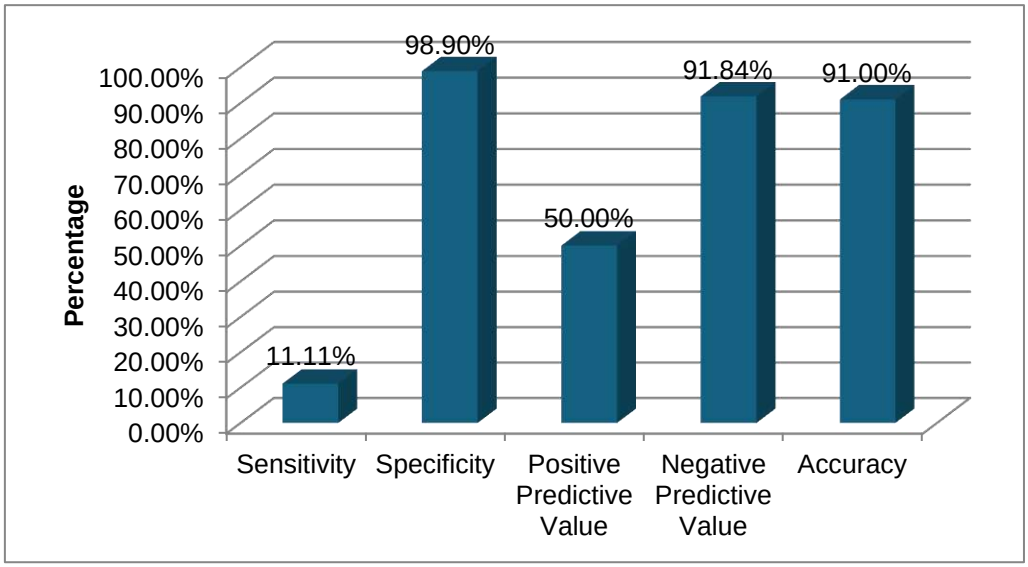

Fig 10: Results for Gram positive obtained on AI device

Table 12: Results for both gram positive and negative(dual infection) obtained on AI device

- For Both Gram Negative and Gram Positive (dual infection)

	Gram Negative and Gram Positive	
Statistic	Value	95% CI
Sensitivity	100.00%	29.24% to 100.00%
Specificity	94.85%	88.38% to 98.31%
Positive Likelihood Ratio	19.4	8.26 to 45.56
Negative Likelihood Ratio	0	
Disease prevalence	3.00%	0.62% to 8.52%
Positive Predictive Value	37.50%	20.35% to 58.49%
Negative Predictive Value	100.00%	96.07% to 100.00%
Accuracy	95.00%	88.72% to 98.36%

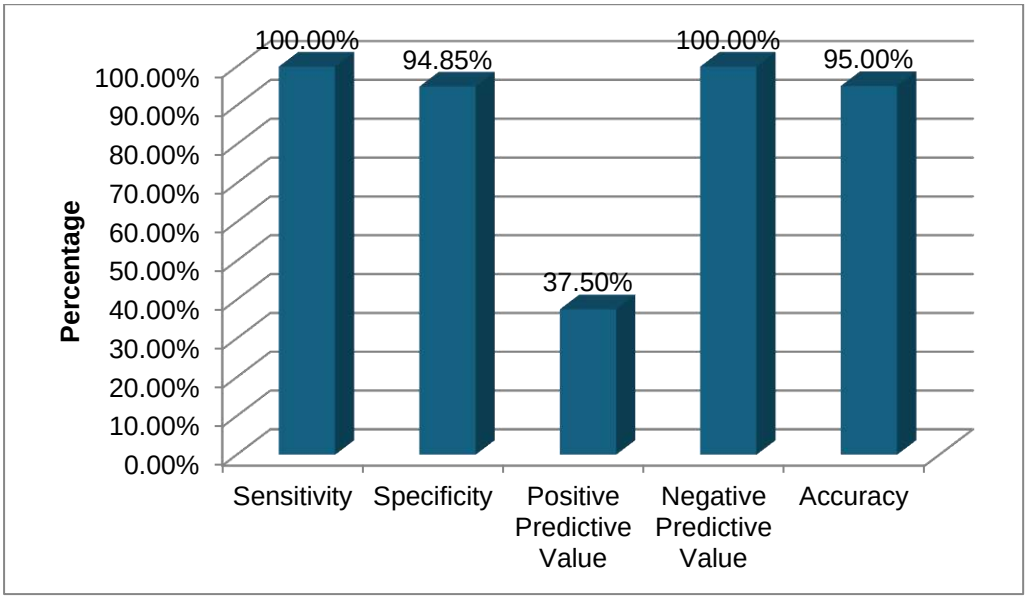

Fig 11: Results for both gram positive and negative (dual infection) obtained on AI device

Sensitivity of both gram positive and negative (dual infection) is 100% (confidence interval- 29.24%- 100%) as compared to tissue culture report and specificity is 94.85 % (confidence interval – 88.38-98.31%).

The overall accuracy of both gram positive and gram negative (dual infection) is 95% (confidence interval- 88.72-98.36%).

Table 13: Results for no growth obtained on AI device

For No Growth:

	No growth	
Statistic	Value	95% CI
Sensitivity	25.00%	7.27% to 52.38%
Specificity	95.24%	88.25% to 98.69%
Positive Likelihood Ratio	5.25	1.46 to 18.86
Negative Likelihood Ratio	0.79	0.59 to 1.05
Disease prevalence	16.00%	9.43% to 24.68%
Positive Predictive Value	50.00%	21.78% to 78.22%
Negative Predictive Value	86.96%	83.34% to 89.88%
Accuracy	84.00%	75.32% to 90.57%

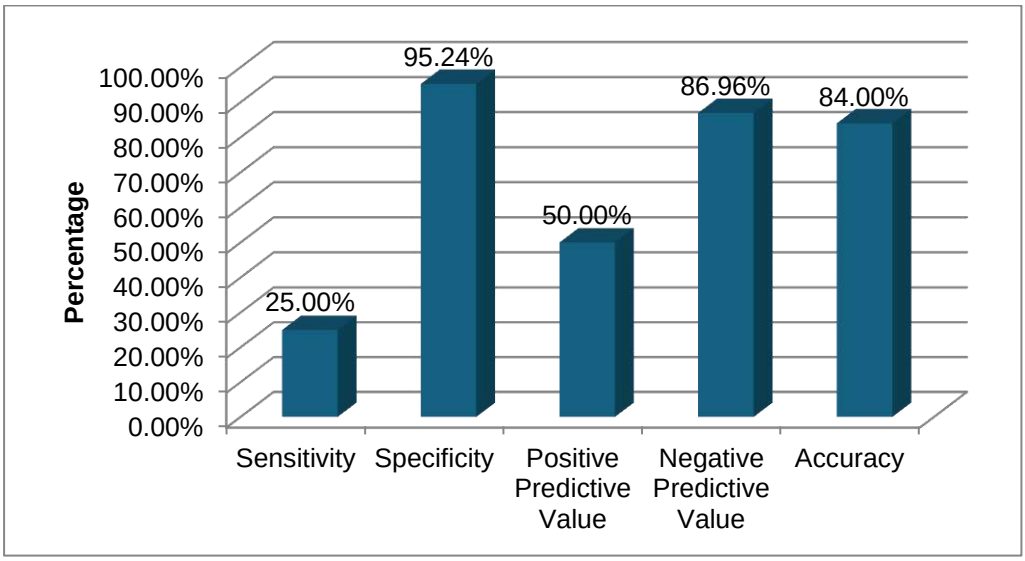

Fig 12: Results for no growth obtained on AI device

Sensitivity of no growth is 25 % (confidence interval- 7.27-52.38 %) as compared to tissue culture report and specificity is 95.24 % (confidence interval – 88.25-98.69%). The overall accuracy of no growth is 84% (confidence interval- 75.32-90.57%).

Table 14: Results for overall AI device

Statistic	Value	95% CI
Sensitivity	80.95%	70.92% to 88.70%
Specificity	25.00%	7.27% to 52.38%
Positive Likelihood Ratio	1.08	0.80 to 1.46
Negative Likelihood Ratio	0.76	0.29 to 1.98
Disease prevalence	84.00%	75.32% to 90.57%
Positive Predictive Value	85.00%	80.74% to 88.45%
Negative Predictive Value	20.00%	8.76% to 39.41%
Accuracy	72.00%	62.13% to 80.52%

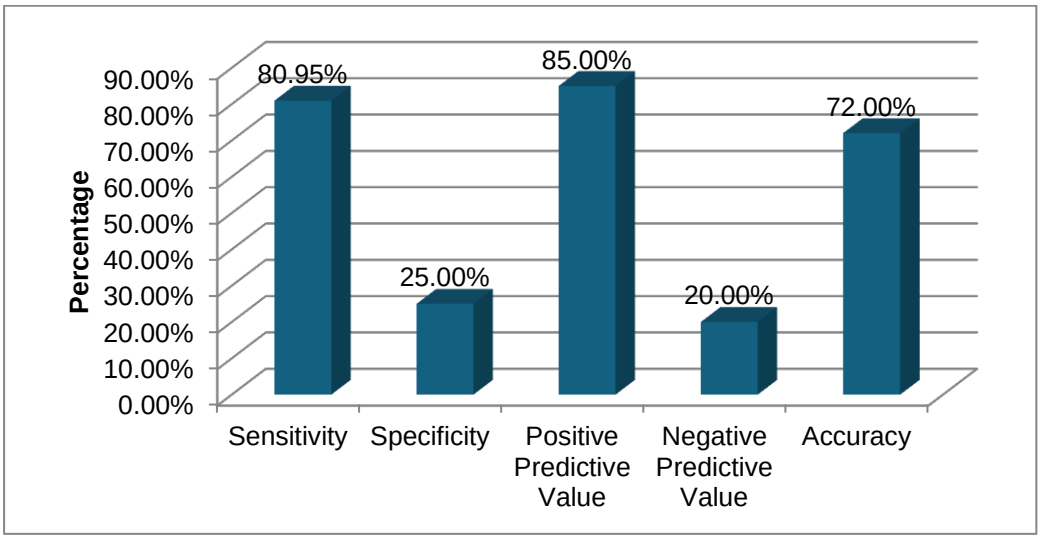

Figure 13: Results for overall AI device

Overall AI device sensitivity 80.95 % (confidence interval- 70.92-88.70%) and specificity is 25 % (confidence interval – 7.27-52.38%) as compared to tissue culture report. The overall accuracy AI device is 72% (confidence interval- 62.13-80.52%).

The agreement between the Illuminate® device and tissue culture methods was assessed using Cohen's kappa statistic and its associated p-value. While the p-value was significant ($p < 0.05$), indicating a statistically significant relationship, the kappa value was 0.287, suggesting only fair agreement between the two methods. This discrepancy highlights that while the relationship between the methods is unlikely to be due to chance, the overall level of agreement is not strong. Further investigation into the sources of disagreement between the methods may be warranted.

Fig 14 to 44 Images captured on the Illuminate® device showing green as gram negative and red as gram positive.

Fig 14: Post debridement DFU showing gram negative growth

Fig 15: Post debridement DFU showing both gram positive and negative growth as red and green respectively

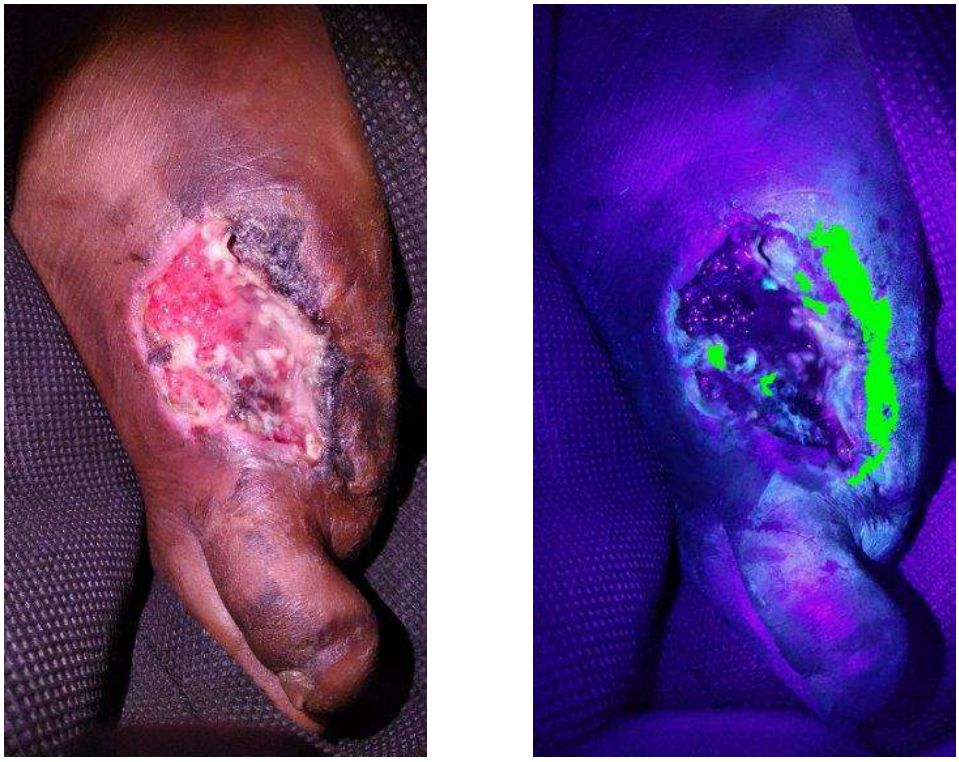

Fig 16: Post debridement DFU showing gram negative growth

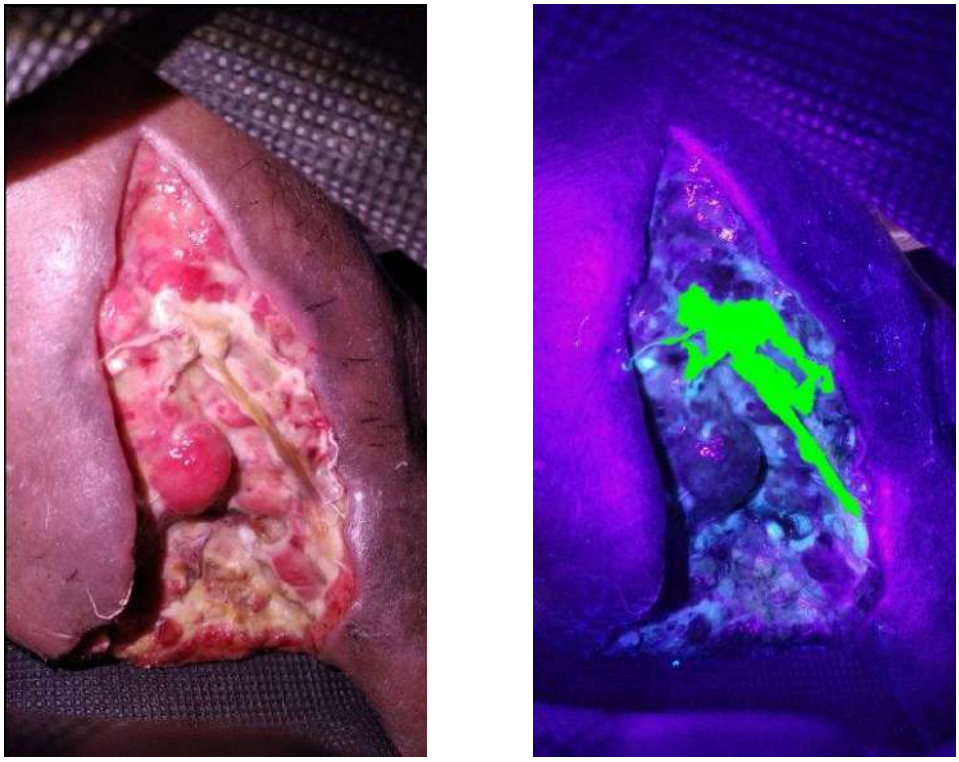

Fig 17: Post debridement DFU showing gram negative growth

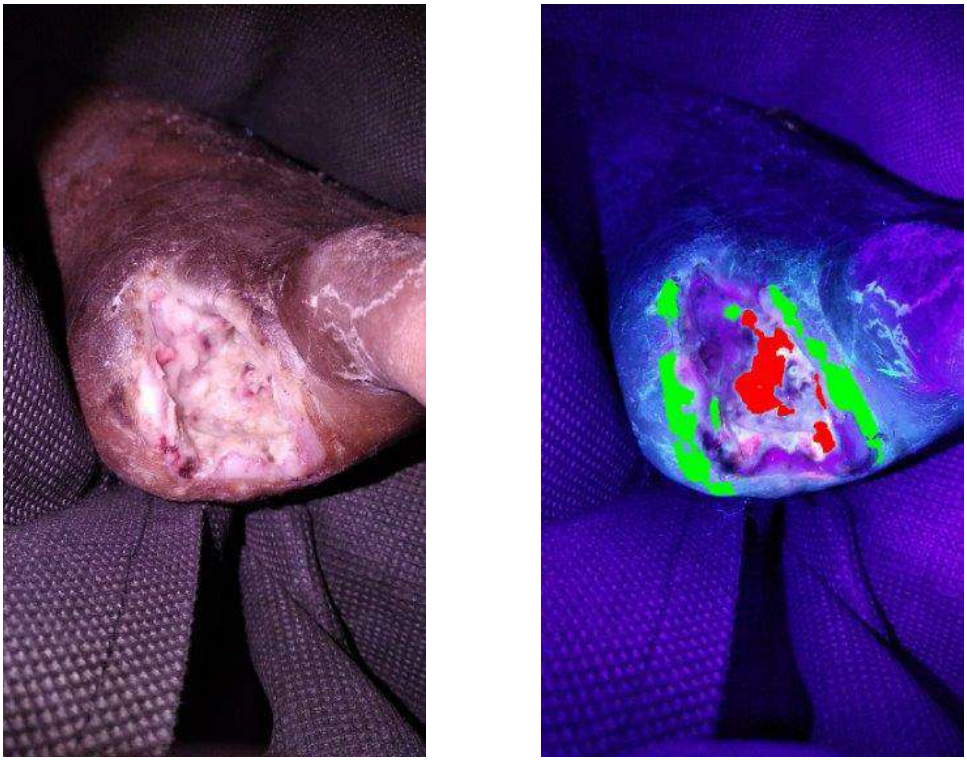

Fig 18: Post debridement DFU showing gram negative growth

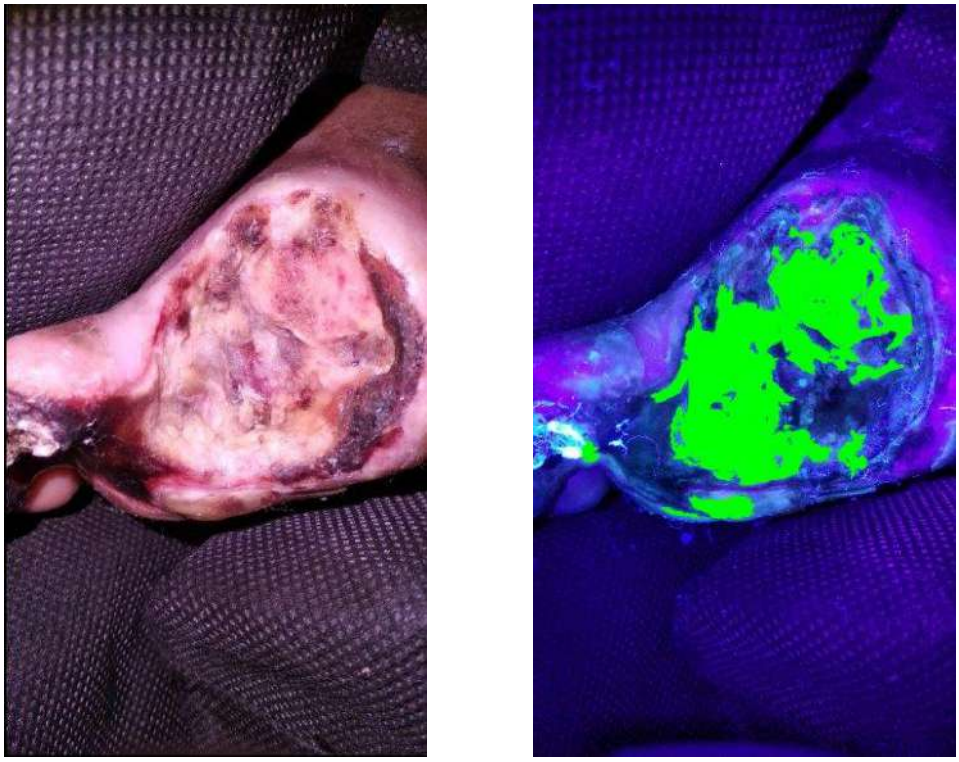

Fig 19: Post debridement DFU showing gram negative growth

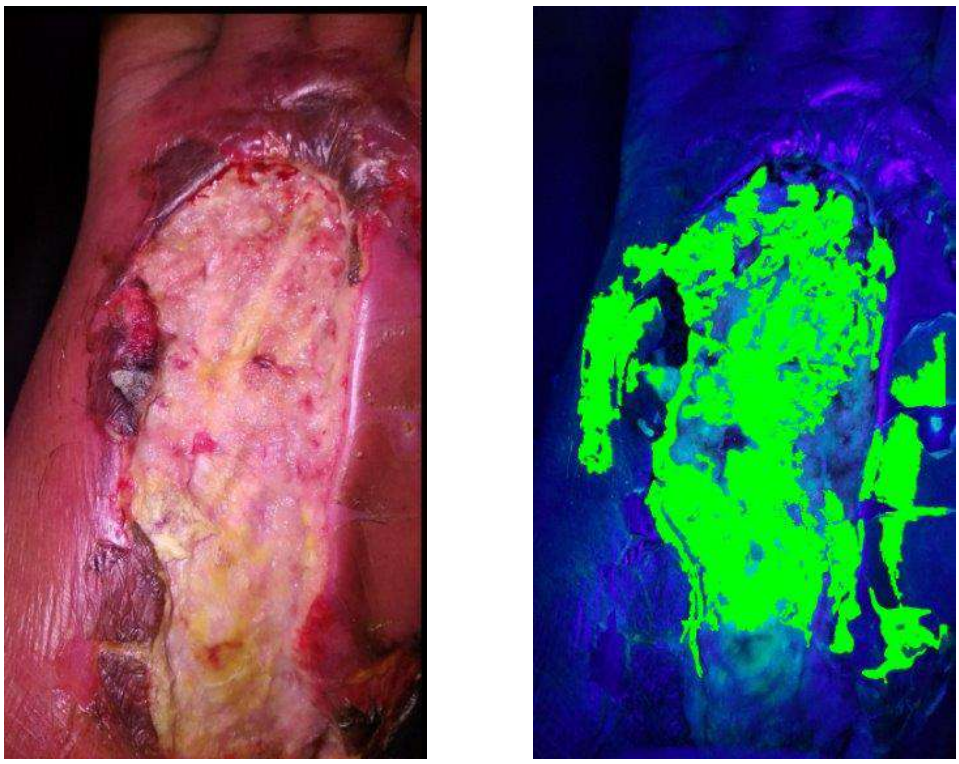

Fig 20: Post debridement DFU showing gram negative growth

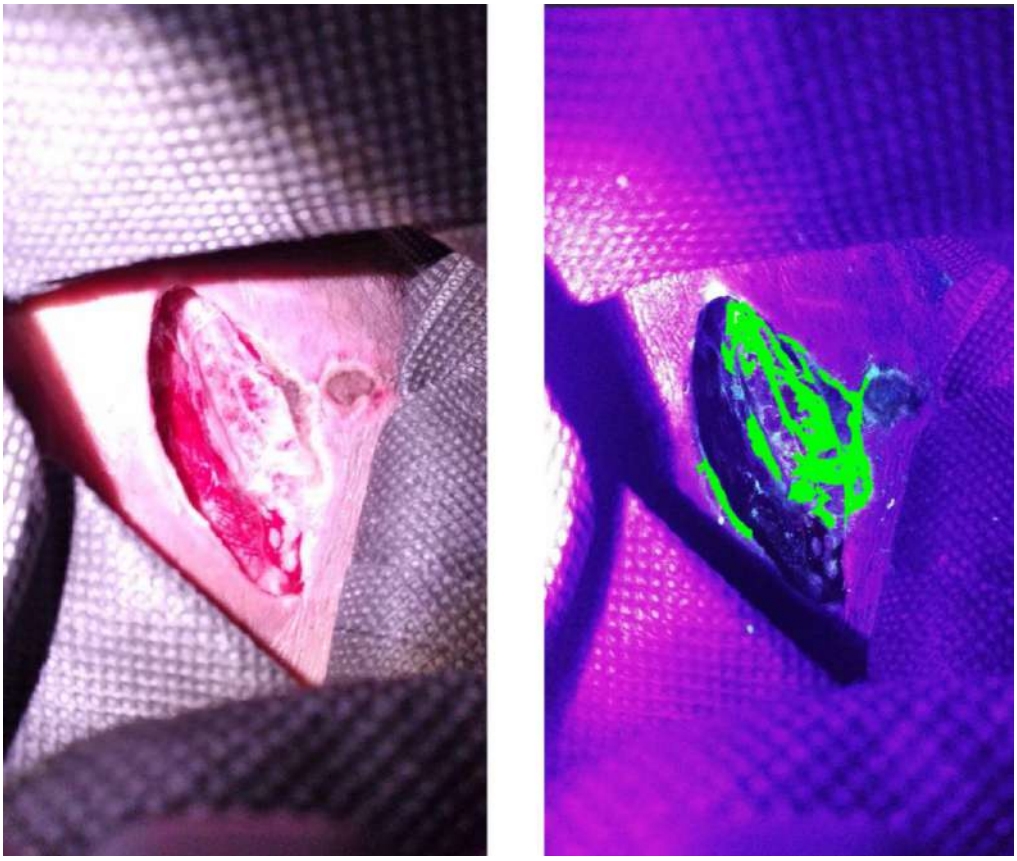

Fig 21: Post trauma debrided wound at thigh showing Gram negative growth

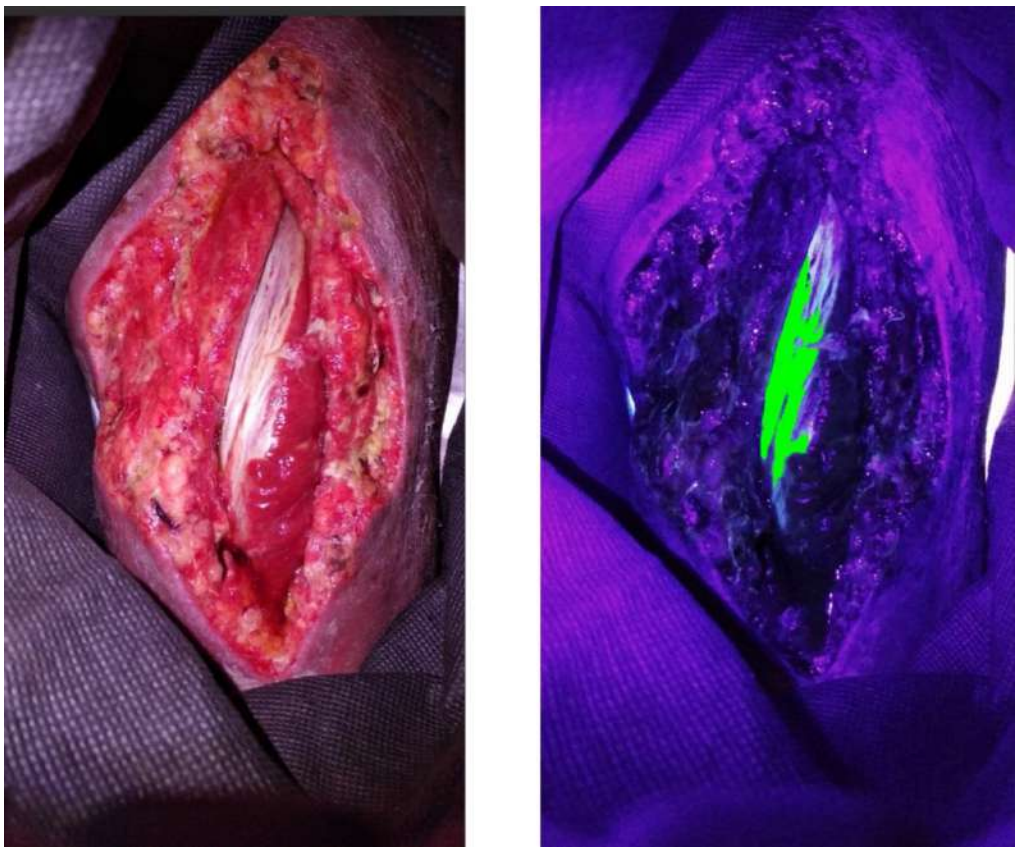

Fig 22: Post trauma thigh debridement showing gram negative growth at tendon site

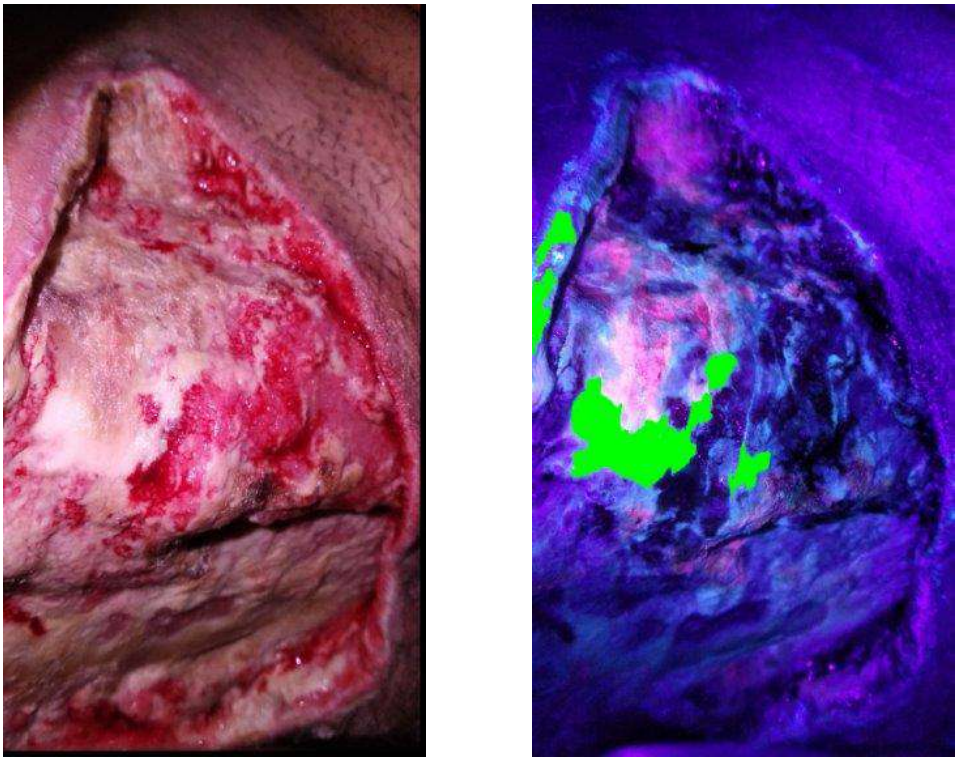

Fig 23: Post traumatic thigh wound after debridement showing Gram negative growth

Fig 24: Post debridement perineal wound showing gram negative growth

Fig 25: Post debridement perineal wound showing gram positive growth

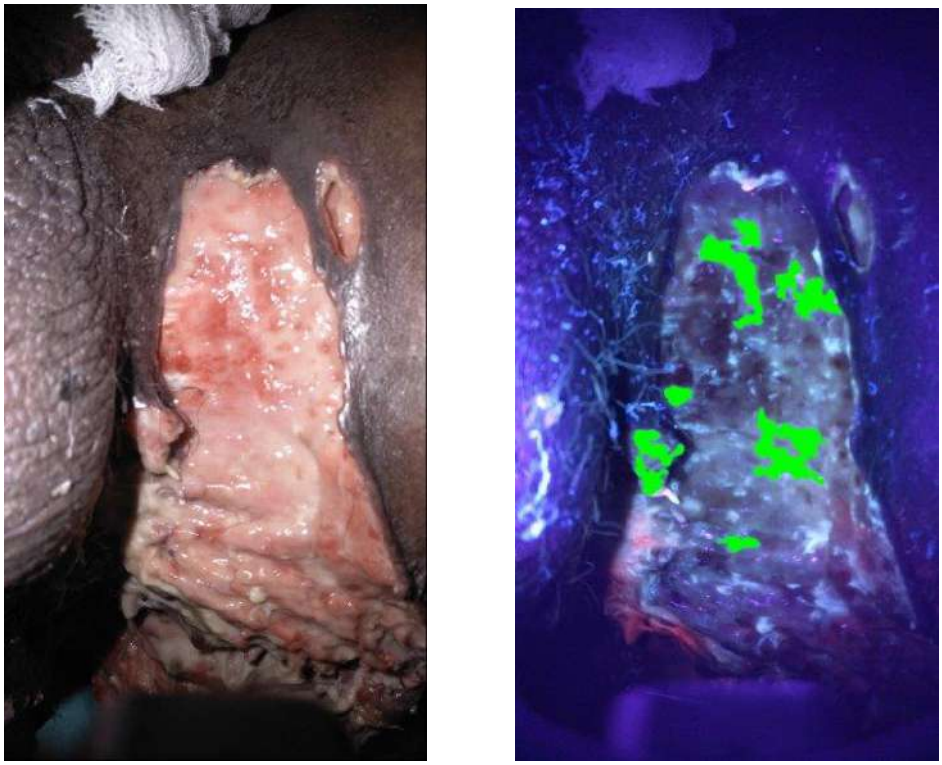

Fig 26: Post debridement perineal abscess showing Gram negative growth

Fig 27: Post incision and drainage of gluteal abscess showing gram negative growth

Fig 28: Post debridement gluteal abscess showing no growth

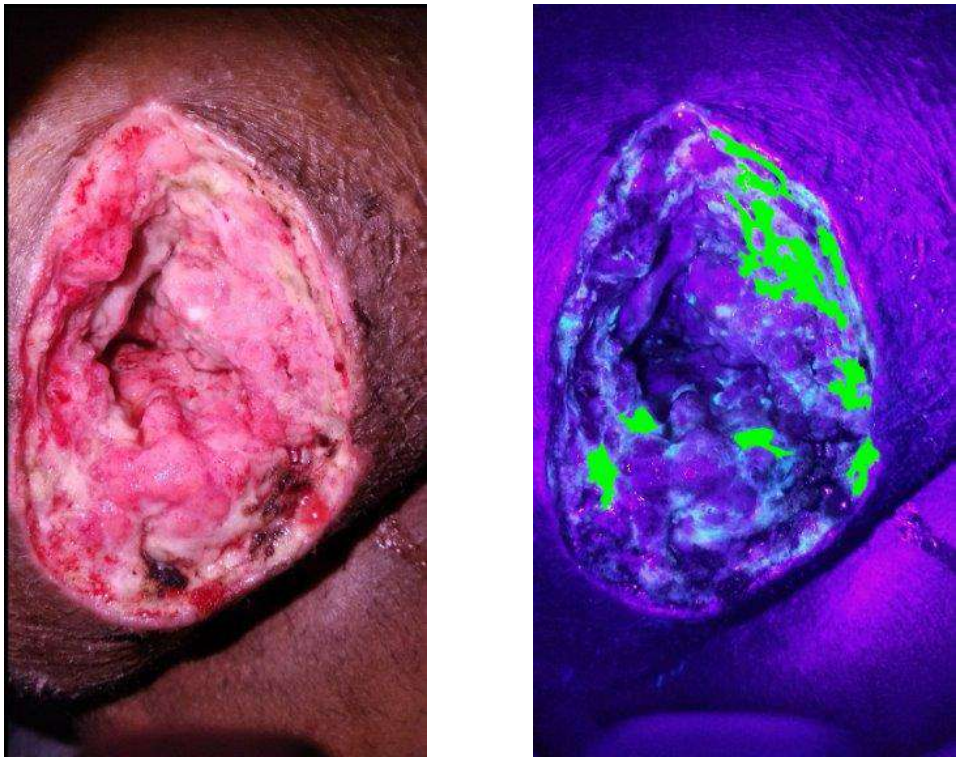

Fig 29: Post debridement Gluteal abscess wound showing Gram Negative growth

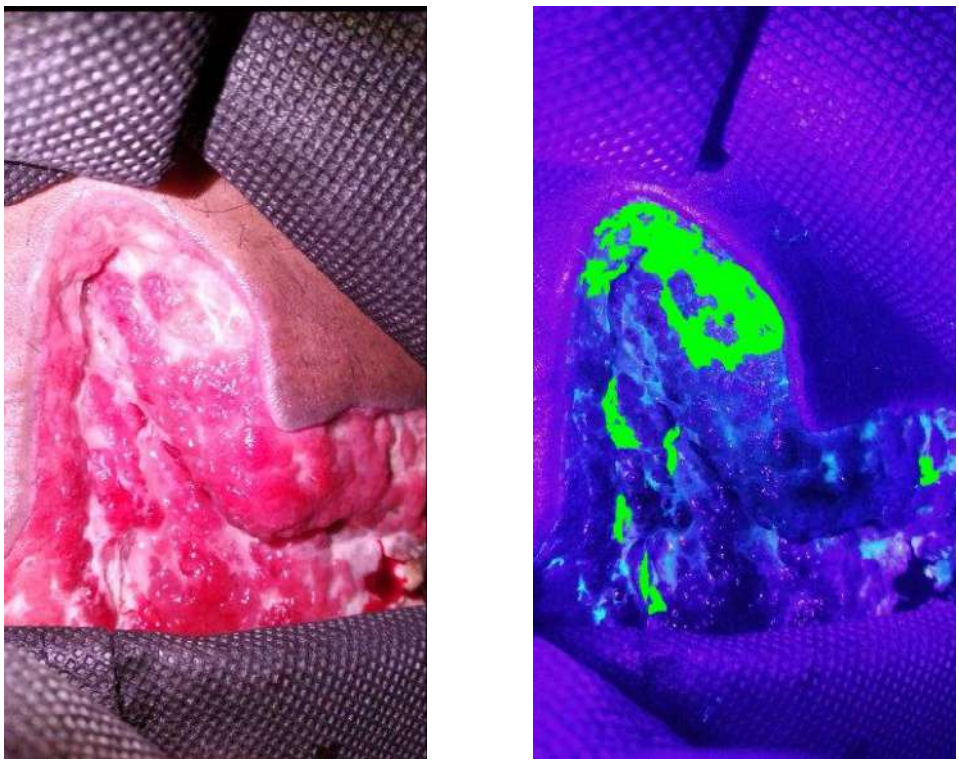

Fig 30: Post incision and drainage gluteal abscess showing Gram Negative growth

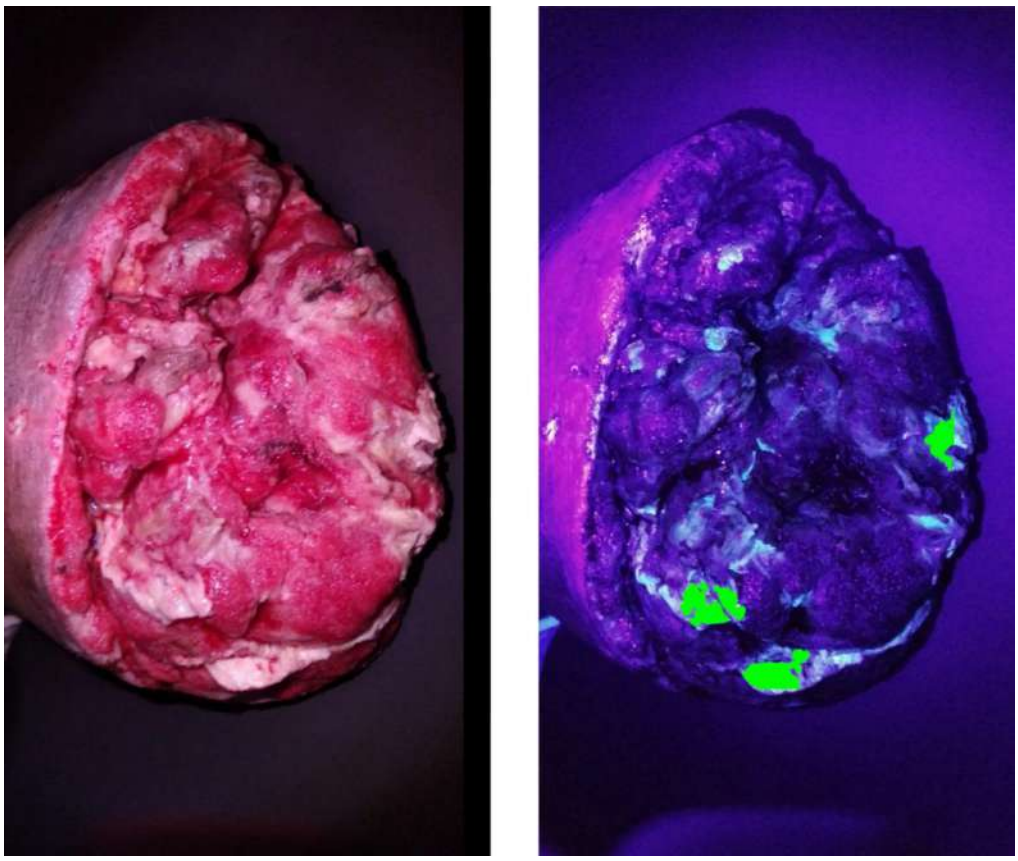

Fig 31: Post below knee amputation stump showing gram negative growth

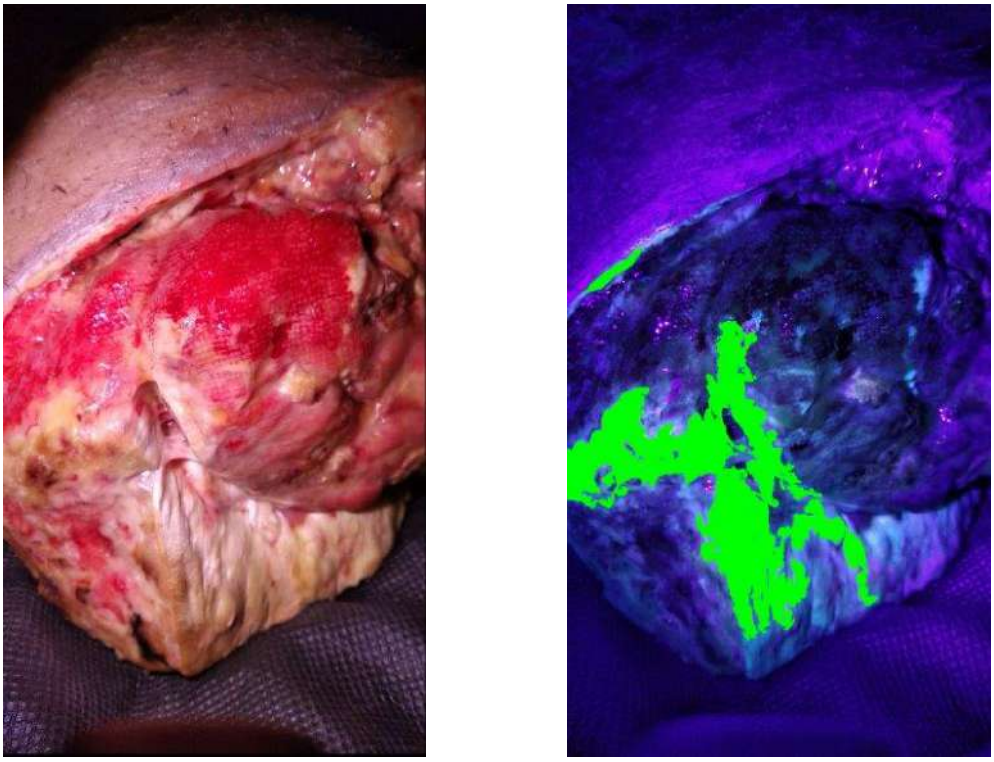

Fig 32: Post op below knee amputation stump showing Fulminant gram negative infection

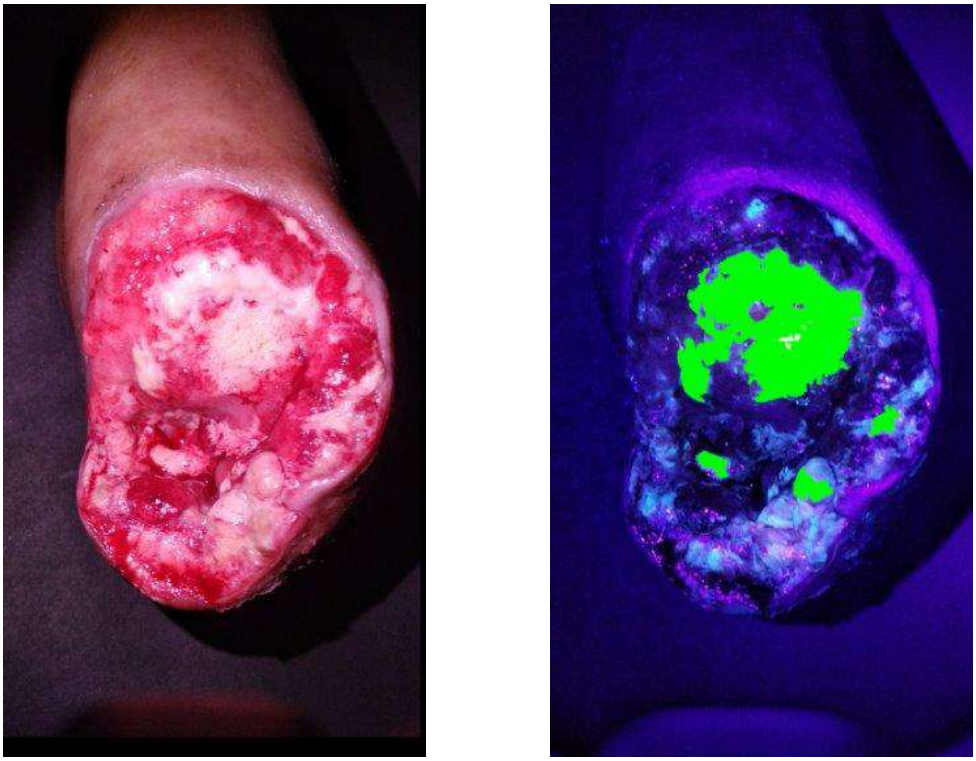

Fig 33: Post below knee amputation stump showing Gram negative infection

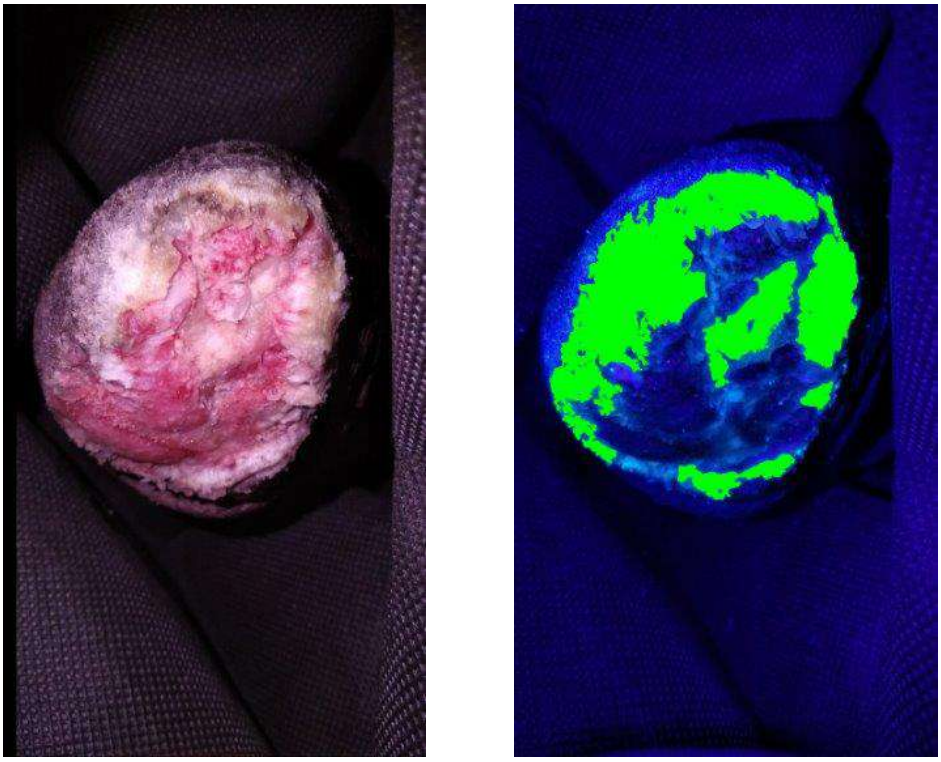

Fig 34: Post op below knee amputation stump showing Fulminant gram negative infection

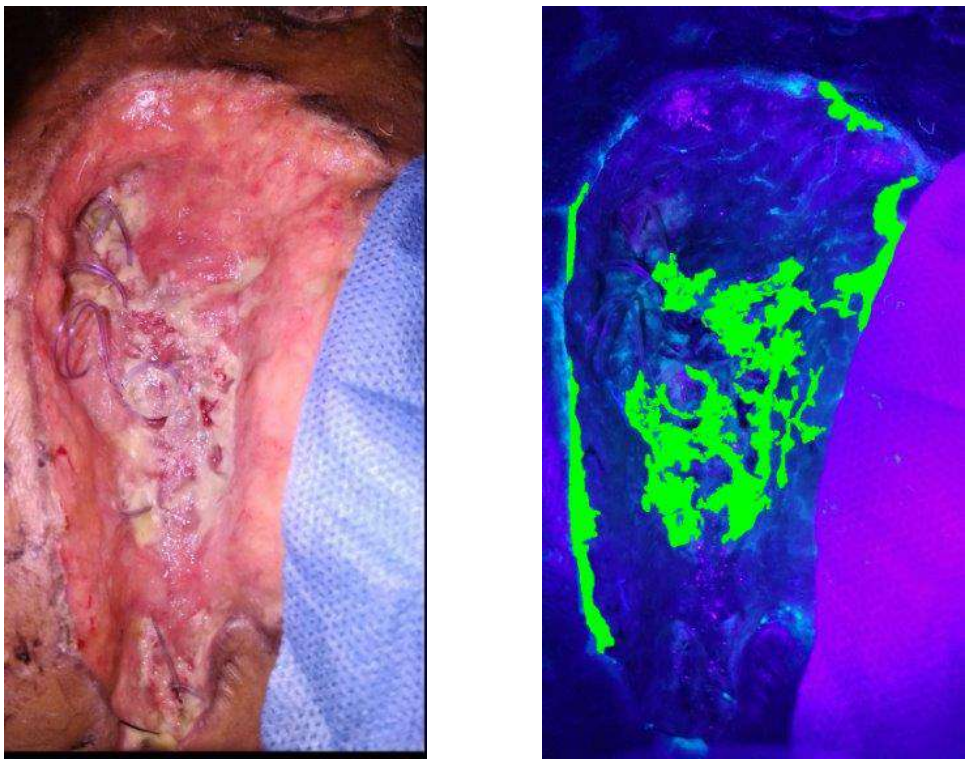

Fig 35: Post op Mid-line Laparotomy wound showing surgical site infection with Gram Negative infection as Green florescence

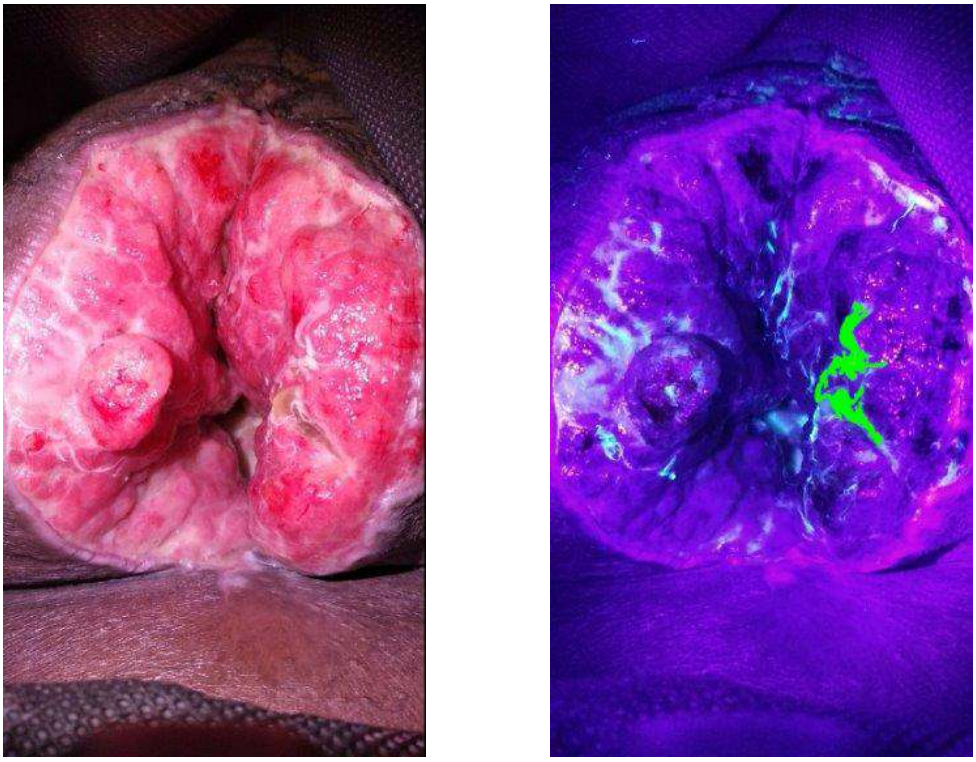

Fig 36: Post Laparotomy Mid line wound showing Gram negative infection

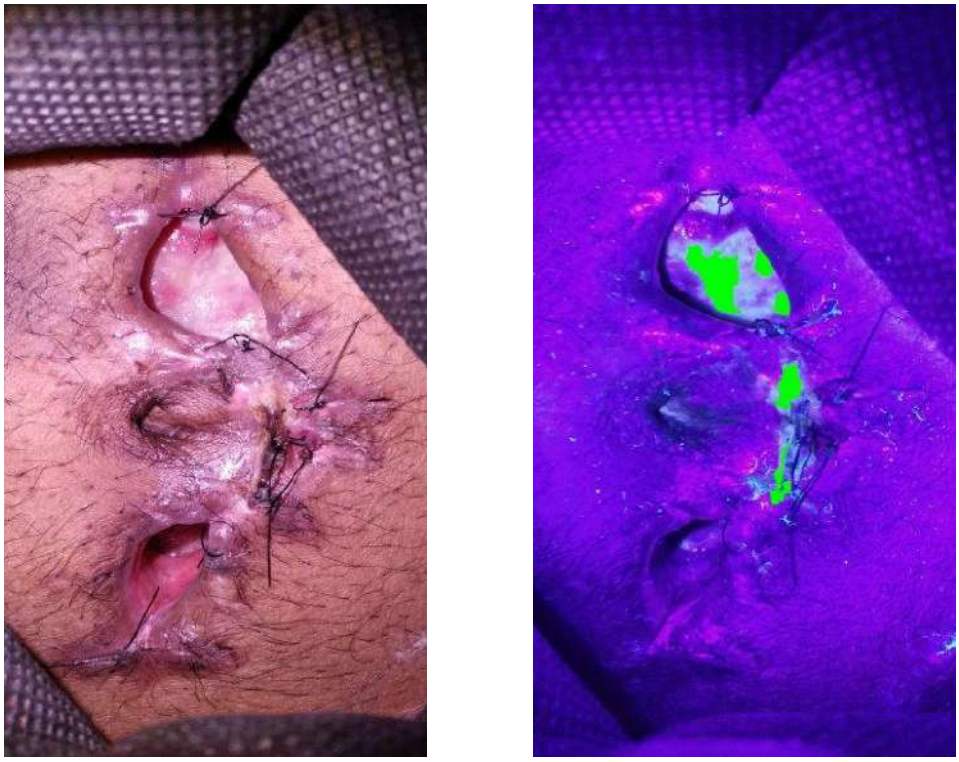

Fig 37: Post Laparotomy Mid line wound showing Gram negative infection

Fig 38: Post Laparotomy Mid line wound showing Gram negative infection

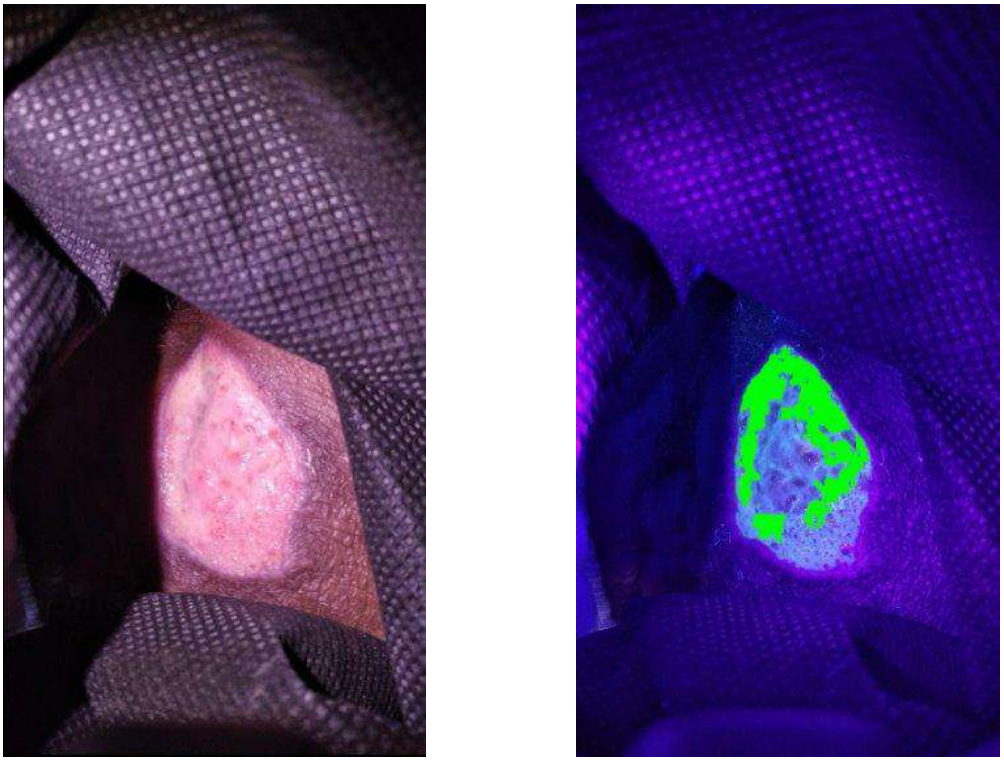

Fig 39: Pre-debridement Venous Ulcer showing Gram Negative growth

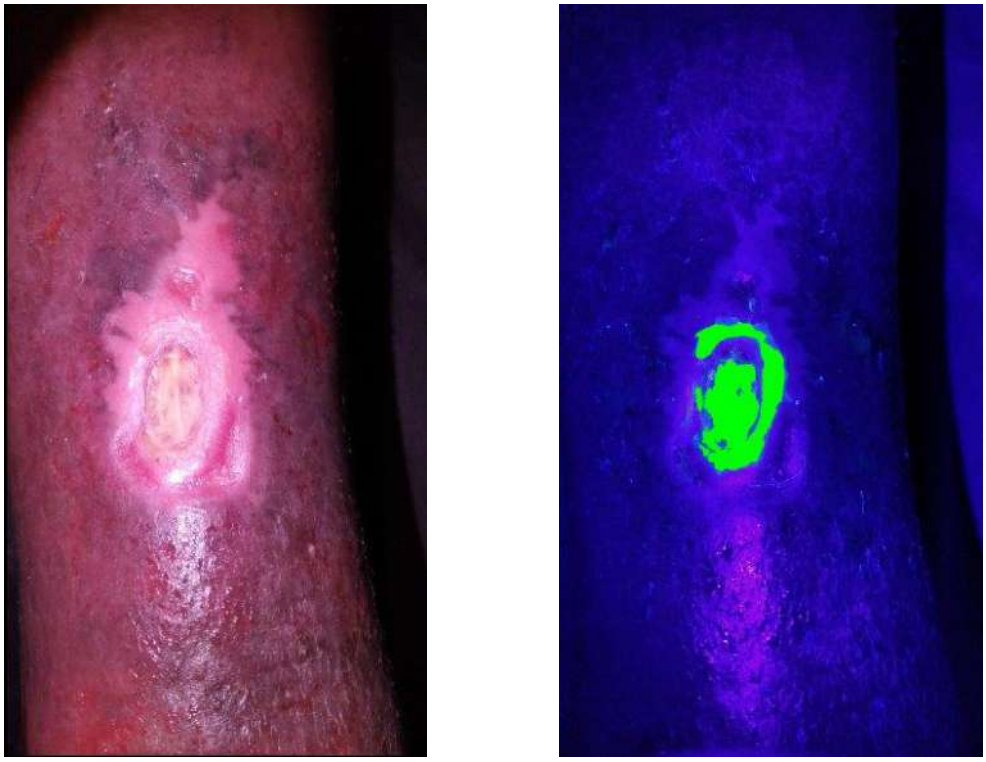

Fig 40: Pre-debridement Venous Ulcer showing Gram Negative growth

Fig 41: Predebridement Decubitus Ulcer at sacral region showing Gram negative growth

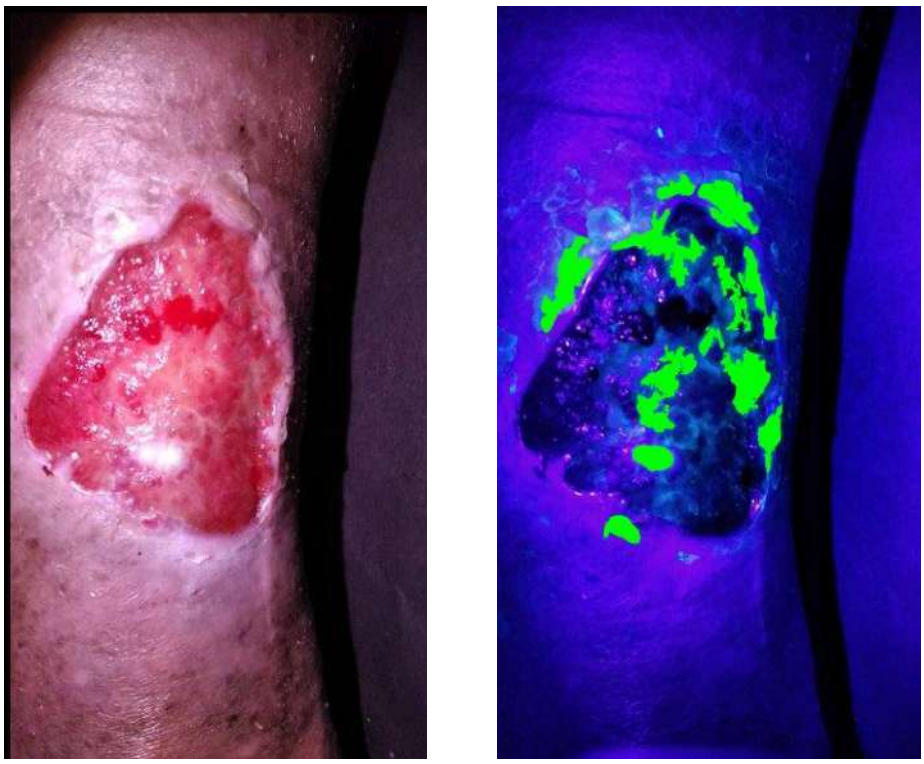

Fig 42: Pre-debridement Venous Ulcer showing Gram Negative growth

Fig 43: Neck abscess showing gram negative growth

Fig 44: Spontaneous eruption at back showing gram negative etiology in a diabetic patient

DISCUSSION

The bacterial load in wounds is often underestimated, leading to an underreporting of infection rates in the wound care population and hence not timely treated. Until this point in time, wound infections have been identified through the observation of clinical signs and symptoms. Four signs of infection—purulent discharge, inflammation, hypergranulation, and erythema—fell below the line of chance, indicating that they are subjective and ineffective in distinguishing between infected and uninfected wounds at threshold bacterial count levels [41].

According to guidelines, intervention is advised in wounds when bacterial colonisation turns into a local infection (10^6 CFU/g) [42]. Addressing the infection at this critical stage helps prevent further escalation along the infection continuum and reduces potential damage to host tissue. This Autofluorescence-based device Illuminate® is a point-of-care device that can detect wound infection with bacterial load 10^4 CFU/g [43].

The device leverages the inherent property of bacterial autofluorescence to detect bioburden in wounds. The native fluorescence in bacteria is attributed to aromatic amino acids such as tyrosine, tryptophan, and phenylalanine, metabolic markers such as NAD(P)H, and Flavins, and markers released during the infectious phase such as porphyrin and pyoverdine [44]. The Illuminate® device demonstrated promising results in detecting bacterial presence in wound ulcers, particularly for Gram-negative bacteria. The high sensitivity (88.89%) for Gram-negative bacteria suggests that the device is effective at identifying these infections. However, the low specificity (35.71%) indicates a tendency for false positives, which could lead to over treatment if not interpreted cautiously. For Gram-positive bacteria, the device showed high specificity (98.90%) but low sensitivity (11.11%). This suggests that while the device may miss some Gram-positive infections, when it does indicate their presence, it is likely to be accurate. The device's performance in identifying cases

with no bacterial growth was characterised by very high specificity (95.24%) but low sensitivity (25.00%). This implies that while the device rarely misidentifies uninfected wounds as infected, it may not reliably detect all uninfected wounds.

Overall predictability for the AI device being 85% positive predictive value and 20% negative predictive value. The overall accuracy of 72%, sensitivity 80.95 % and specificity is 25% suggests that while the Illuminate® device is a promising tool, but there is room for improvement.

The device's performance could potentially be enhanced through further refinement of its algorithms or by combining its results with clinical assessment.

Various studies have explored the use of autofluorescence properties exhibited by these pathological organisms for differentiation. Ongoing research is focused on harnessing the differential quantitative property of auto fluorescence by feeding 5-aminolevulinic acid to wounds. which serves as a precursor to fluorescent molecules required by bacterial pathogens. This leads to the amplification of fluorescence signals, thereby confirming the presence of infection within the wound.

With more information and research on this topic, more differential patterns of infections can be identified in particular variants and strains of bacteria. Fluorescence-based photography might in the future replace the tissue culture method to some extent with instant results. It can be advantageous in a variety of settings, starting from operating theatres to verify the presence or absence of any remaining infection, to the monitoring of sterilization processes.

The implications of this autofluorescence based instant detection are numerous.The differentiation and instant identification of non-infected wounds would serve to prevent the unethical and indiscriminate use of antibiotics, even in instances of non-infected wounds.This device is capable of real-time usage in the operating theatre during wound debridement. It can be utilised to detect any remaining infected tissue that needs to be removed before closure,

providing immediate results.Furthermore, it can function as a preventive screening tool in wound management. It can determine the necessity for interventions such as wound debridement or antibiotic therapy.

If wound is uninfected, it is beneficial to exercise caution and avoid indiscriminate antibiotic use when unnecessary, thus contributing to the reduction of antibiotic resistance within the community.

Limitations of the study:

The results of our study came out to be expected except for the lower detection of gram positive infected wounds, for which the calculated results are statistically insignificant for a low incidence of gram positive infections. The study can be improved with a large sample size statistically enough for all strains of infection, to properly test the device and its autofluorescence property.

The limiting factor is superficial contamination of the wound can alter the result of the device. Also, Bone visualisation in ankle and shin wounds can alter the results of the device.

The device has limitations in detecting deep-seated bacterial infections and distinguishing pathogenic bacteria from commensal ones. While it can provide an estimate of significant bacterial load, accurate quantification still necessitates tissue culture. Additionally, the device does not replace culture and sensitivity tests, which are crucial for selecting appropriate antibiotics, especially in the context of drug resistance. To enhance the device's sensitivity and specificity, incorporating additional fluorescence patterns is needed.

SUMMARY AND CONCLUSION

This study evaluated the efficacy of the Illuminate® device, a novel autofluorescence-based artificial intelligence tool, in detecting and classifying bacterial infections in wound ulcers. With recent advances and by using the property of differential spectrum of absorption and reflection of UV light, causative organisms can be identified instantly. The property of autofluorescence has made early antimicrobial intervention possible. Though tissue culture from the wound/Infection remains the gold standard, these AI-based devices can be helpful in remote areas for early management of infections. The reliability and accuracy of these devices in diagnosing all spectra of microorganisms are an area of research.

The present study has validated the role of autofluorescence in the detection of gram-negative bacteria however we could not validate the results for gram-positive organisms.The device showed high sensitivity for Gram-negative bacteria, high specificity for Gram-positive bacteria, and high specificity for identifying uninfected wounds.

In conclusion, the Illuminate® device demonstrates potential as a rapid, non-invasive tool for bacterial detection in wound ulcers. Its high sensitivity for Gram-negative bacteria and high specificity for Gram-positive bacteria make it a valuable adjunct to clinical assessment. However, its limitations, particularly in terms of specificity for gram-negative bacteria and sensitivity for uninfected wounds, should be considered in its clinical application.

Future research directions could include larger, multi-center studies to validate these findings and further investigate the device's performance across a wider range of wound types. Additionally, studies examining the impact of using the device on clinical outcomes and antibiotic stewardship would be valuable in assessing its practical benefits in wound care management. Finally, efforts should be directed towards further refinement of the device's algorithms to improve its overall accuracy, potentially enhancing its utility as a diagnostic tool in clinical settings.

The Illuminate® device represents a promising step towards rapid, point-of-care diagnosis of wound infections, potentially improving wound management and patient outcomes. Consequently, there exists a significant opportunity for enhancement in the detection of additional fluorescence biomolecules for the differential diagnosis of various infections and the utilisation of bacterial feeding fluorescence molecule precursors to enhance fluorescence as a means of confirmation. These specific areas necessitate further research to fully utilise the auto fluorescence property in the rapid detection and management of wounds.

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Serial No.	Name	Illuminate ID	Hospital ID	Age	Gender	Wound Location	Wound Aetiology	Exudate Type	Exudate Amount	Actual Visit #	Device Visit #	Visit Type	Illuminate Result	Tissue Sample Result	Interpretation
1	SK	ADDBH01_4	1392456	67	F	Foot	Diabetic	None	None	2	2	OPD	Gram Negative	Klebsiella Oxytoca	Correct
2	RM	ADDBH01_13	1298884	40	M	Foot	Diabetic	Purulent	Scanty	1	1	Ipd	Gram Negative	Staphylococcus aureus	Incorrect
3	MS	ADDBH01_15	1321553	50	M	Foot	Diabetic	None	None	30	30	OPD	Gram Negative and Gram Positive	Escherichia coli	Incorrect
4	SS	ADDBH01_22	1345678	63	M	Leg	Diabetic	None	None	23	23	OPD	Gram Negative	Escherichia coli	Correct
5	PJ	ADDBH01_48	1542862	58	M	Foot	Diabetic	Purulent	Scanty	30	30	OPD	Gram Negative	Escherichia coli	Correct
6	Jsb	ADDBH01_77	1327506	68	M	Leg	Traumatic	None	None	11	11	Follow up	No Growth	Acinetobacter baumannii	Incorrect
7	PS	ADDBH01_86	1423897	51	M	Foot	Diabetic	None	None	1	1	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
8	GC	ADDBH01_94	1350988	61	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative	Staphylococcus haemolyticus	Incorrect
9	Ps	ADDBH01_96	1345507	53	M	Leg	Other	None	None	2	2	Ipd	No Growth	Klebsiella pneumoniae	Incorrect
10	AG	ADDBH01_97	514054	18	M	Perianal Region	Diabetic	None	None	1	1	Ipd	Gram Negative	Klebsiella pneumoniae	Correct
11	KK	ADDBH01_99	1483206	74	M	Foot	Diabetic	None	None	4	4	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
12	KK	ADDBH01_104	1345918	50	F	Leg	Venous ulcer	None	None	1	2	Ipd	Gram Negative	Escherichia coli	Correct
13	SS	ADDBH01_116	1364734	55	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative and Gram Positive	Enterococcus faecalis, Escherichia coli	Correct
14	KKG	ADDBH01_117	1347265	72	M	Foot	Diabetic	None	none	27	27	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
15	Sn	ADDBH01_121	1373119	75	M	Hand	Traumatic	None	None	1	1	Ipd	No Growth	Pseudomonas aeroginosa, Providencia stuartii	Incorrect
16	AM	ADDBH01_132	1597342	50	F	Foot	Diabetic	None	None	2	2	OPD	No Growth	No growth	Correct
17	KS	ADDBH01_133	1373998	56	M	Abdominal	Surgical Site Infection	None	none	1	1	Ipd	Gram Negative	Escherichia coli	Correct
18	BM	ADDBH01_142	1390613	40	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative	Acinetobacter baumannii	Correct
19	V	ADDBH01_145	1387549	43	M	Abdominal	Surgical Site Infection	Purulent	Minimal	1	1	Ipd	No Growth	Klebsiella pneumoniae	Incorrect
20	SS	ADDBH01_150	1393754	50	M	Leg	Traumatic	None	None	1	1	Ipd	Gram Negative	Klebsiella pneumoniae, Acinetobacter baumannii	Correct
21	Ks	ADDBH01_154	1406590	60	M	Foot	Venous ulcer	None	None	1	1	Ipd	Gram Negative	Pseudomonas aeruginosa, Escherichia coli	Correct
22	R	ADDBH01_158	1409633	24	M	Hand	Cellulitis	None	None	1	1	Ipd	No Growth	No growth	Correct
23	CS	ADDBH01_160	1325897	44	M	Thigh	Diabetic	None	None	1	1	OPD	No Growth	No growth	Correct
24	Ks	ADDBH01_161	1408467	80	M	Gluteal Region	Diabetic	None	None	1	1	Ipd	Gram Negative	Escherichia coli	Correct
25	RK	ADDBH01_165	1415174	50	M	Leg	Diabetic	Serous	None	3	3	Ipd	Gram Negative	Klebsiella pneumoniae, Enterobacter aerogenes	Correct

Serial No.	Name	Illuminate ID	Hospital ID	Age	Gender	Wound Location	Wound Aetiology	Exudate Type	Exudate Amount	Actual Visit #	Device Visit #	Visit Type	Illuminate Result	Tissue Sample Result	Interpretation
26	RR	ADDBH01_169	1380383	40	F	Abdominal	Surgical Site Infection	None	None	1	1	Ipd	Gram Negative	No growth	Incorrect
27	RK	ADDBH01_170	155077	77	M	Foot	Diabetic	None	None	8	8	OPD	Gram Negative	Escherichia coli	Correct
28	HS	ADDBH01_172	1425847	60	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative	Enterobacter cloacae	Correct
29	AD	ADDBH01_173	1426977	58	M	Gluteal Region	Pressure Sore	None	None	1	1	Ipd	Gram Positive	Escherichia coli, Pseudomonas aeruginosa	Incorrect
30	G	ADDBH01_174	1427184	30	M	Abdominal	Surgical Site Infection	None	None	1	1	Ipd	Gram Negative	No growth	Incorrect
31	Js	ADDBH01_176	1394776	53	M	Abdominal	Surgical Site Infection	Purulent	Copious	1	1	Ipd	No Growth	No growth	Correct
32	JS	ADDBH01_180	1431184	27	M	Perianal Region	Traumatic	Purulent	Scanty	1	1	Ipd	Gram Negative	Escherichia coli	Correct
33	RPV	ADDBH01_193	1378956	56	M	Back	Diabetic	None	None	14	14	OPD	Gram Negative and Gram Positive	Enterococcus feacium	Incorrect
34	BS	ADDBH01_194	1451098	30	M	Gluteal Region	Pressure Sore	None	None	2	2	Follow up	Gram Negative	Escherichia coli	Correct
35	RB	ADDBH01_199	1395865	29	F	Abdominal	Surgical Site Infection	Purulent	Copious	1	1	Follow up	Gram Negative	Klebsiella pneumoniae, Pseudomonas aeruginosa	Correct
36	VJ	ADDBH01_200	1325645	53	F	Foot	Diabetic	None	None	7	7	Ipd	Gram Negative and Gram Positive	Staphylococcus haemolyticus	Incorrect
37	H	ADDBH01_202	1512345	81	M	Foot	Diabetic	Purulent	Scanty	9	9	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
38	W	ADDBH01_207	1447308	61	F	Leg	Diabetic	None	None	2	2	Ipd	Gram Negative	Escherichia coli	Correct
39	NJ	ADDBH01_208	1271866	56	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative	Pseudomonas aeruginosa	Correct
40	RK	ADDBH01_210	1429731	62	M	Leg	Diabetic	None	None	4	4	Ipd	Gram Negative	Klebsiella pneumoniae	Correct
41	KD	ADDBH01_213	1455441	78	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative	No growth	Incorrect
42	VK	ADDBH01_214	1449712	53	F	Abdominal	Surgical Site Infection	None	None	1	1	Ipd	Gram Negative	No growth	Incorrect
43	Rg	ADDBH01_223	1449982	64	M	Leg	Venous ulcer	None	None	1	1	Ipd	Gram Negative	No growth	Incorrect
44	GS	ADDBH01_224	1523647	50	M	Foot	Pressure Sore	None	None	1	1	Opd	Gram Negative	Proteus vulgaris	Correct
45	HK	ADDBH01_227	643315	65	F	Abdominal	Surgical Site Infection	None	None	1	1	Ipd	Gram Negative	Klebsiella pneumoniae	Correct
46	MK	ADDBH01_229	1456685	56	F	Leg	Venous ulcer	None	none	1	1	Opd	Gram Negative	No growth	Incorrect
47	JK	ADDBH01_231	1460044	50	F	Thigh	Venous ulcer	None	None	1	1	Ipd	Gram Negative	No growth	Incorrect
48	VK	ADDBH01_232	1467254	38	M	Foot	Diabetic	None	None	1	1	Opd	Gram Negative	Klebsiella pneumoniae	Correct
49	KK	ADDBH01_238	1471219	62	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative	Klebsiella pneumoniae	Correct

Serial No.	Name	Illuminate ID	Hospital ID	Age	Gender	Wound Location	Wound Aetiology	Exudate Type	Exudate Amount	Actual Visit #	Device Visit #	Visit Type	Illuminate Result	Tissue Sample Result	Interpretation
50	SS	ADDBH01_240	1471774	69	M	Leg	Diabetic	None	None	1	1	Opd	Gram Negative	No growth	Incorrect
51	JS	ADDBH01_241	1462366	45	M	Foot	Diabetic	None	None	1	1	Ipd	Gram Negative	Escherichia coli	Correct
52	KS	ADDBH01_242	230926	47	M	Foot	Venous ulcer	None	None	1	1	Ipd	Gram Negative	Acinetobacter baumannii	Correct
53	BK	ADDBH01_243	323905	59	F	Foot	Maligant	Purulent	Scanty	1	1	Opd	Gram Positive	Staphylococcus hominis	Correct
54	PK	ADDBH01_244	1471346	63	M	Leg	Venous and diabetic	None	None	1	1	Ipd	Gram Negative	Acinetobacter baumannii	Correct
55	RB	ADDBH01_246	1491256	52	M	Foot	Diabetic	None	None	29	29	IPD	Gram Negative	Diptheroids	Incorrect
56	GS	ADDBH01_251	1471317	60	M	Abdominal	Pressure Sore	None	None	1	1	IPD	Gram Negative	Burkholderia cepacia	Correct
57	MJ	ADDBH01_253	1351573	73	M	Foot	Diabetic	None	None	2	2	Ipd	Gram Negative and Gram Positive	Pseudomonas aeruginosa	Incorrect
58	N	ADDBH01_255	1478676	60	F	Back	Pressure Sore	None	None	1	1	Ipd	Gram Negative	Pseudomonas aeruginosa	Correct
59	BS	ADDBH01_256	1480239	49	M	Foot	Diabetic	None	None	1	1	IPD	Gram Negative	Staphylococcus aureus	Incorrect
60	YC	ADDBH01_260	1480744	35	M	Gluteal Region	Pressure Sore	Purulent	Scanty	1	1	Ipd	Gram Negative	Escherichia coli	Correct
61	Sg	ADDBH01_272	1491739	60	F	Chest	Surgical Site Infection	None	None	1	1		Gram Negative	Pseudomonas aeruginosa	Correct
62	CD	ADDBH01_277	1490488	64	M	Abdominal	Surgical Site Infection	None	None	4	4		Gram Negative	Pseudomonas aeruginosa, Acinetobacter baumannii	Correct
63	TS	ADDBH01_286	1488634	63	F	Abdominal	Surgical Site Infection	None	None	1	1	IPD	Gram Negative	Enterococcus faecalis, Escherichia coli	Incorrect
64	G	ADDBH01_289	474022	67	M	Foot	Diabetic	Purulent	Minimal	3	3	OPD	Gram Negative and Gram Positive	Citrobacter koseri ,Enterococcus faecalis	Correct
65	BS	ADDBH01_295	1532478	78	M	Foot	Diabetic	Purulent	Minimal	15	15	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
66	RL	ADDBH01_297	1514885	76	M	Leg	Diabetic	None	none	1	1	OPD	Gram Negative	Escherichia coli	Correct
67	MV	ADDBH01_316	1522327	48	F	Abdominal	Surgical Site Infection	None	None	1	1	IPD	Gram Negative	Acinetobacter baumannii	Correct
68	BM	ADDBH01_317	1425698	52	M	Leg	Cellulitis	None	None	1	1	OPD	Gram Negative	Staphylococcus aureus	Incorrect
69	SJ	ADDBH01_318	1365874	57	F	Foot	Diabetic	None	None	1	1	OPD	Gram Negative	No growth	Incorrect
70	IS	ADDBH01_321	430845	70	M	Foot	Diabetic	None	None	1	1	OPD	Gram Negative	Stenotrophomonas maltophilia	Correct
71	SS	ADDBH01_330	1456287	57	M	Foot	Diabetic	None	None	5	5	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
72	JK	ADDBH01_332	1541651	59	M	Thigh	Diabetic	None	None	7	7	OPD	Gram Negative	Escherichia coli	Correct
73	G	ADDBH01_333	1356784	68	F	Foot	Diabetic	None	None	2	1	OPD	Gram Negative	Escherichia coli	Correct
74	GK	ADDBH01_345	1536217	51	F	Foot	Diabetic	Serous	Minimal	1	1	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
75	KS	ADDBH01_352	1307255	29	M	Thigh	Cellulitis	None	None	4	4	OPD	Gram Negative	Escherichia coli	Correct
76	SR	ADDBH01_353	1457895	83	F	Pilonidal Region	Pilonidal Sinus	Purulent	Minimal	1	1	OPD	Gram Negative	Escherichia coli	Correct

Serial No.	Name	Illuminate ID	Hospital ID	Age	Gender	Wound Location	Wound Aetiology	Exudate Type	Exudate Amount	Actual Visit #	Device Visit #	Visit Type	Illuminate Result	Tissue Sample Result	Interpretation
77	HS	ADDBH01_354	1547231	75	M	Leg	Venous ulcer	None	None	1	1	OPD	Gram Negative	Escherichia coli	Correct
78	PK	ADDBH01_364	1563924	30	M	Perianal Region	Diabetic	None	None	1	1	IPD	Gram Negative	Escherichia coli	Correct
79	A	ADDBH01_368	1489762	53	F	Leg	Diabetic	None	none	1	1	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
80	NS	ADDBH01_384	1345721	60	M	Leg	Venous ulcer	Purulent	Minimal	2	4	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
81	NS	ADDBH01_386	1534687	63	M	Leg	Venous ulcer	None	None	1	1	OPD	Gram Negative	No growth	Incorrect
82	AK	ADDBH01_387	1599261	74	M	Foot	Non Healing Ulcer	Purulent	Scanty	1	1	OPD	Gram Negative	Acinetobacter baumannii	Correct
83	VK	ADDBH01_394	1587632	60	M	Foot	Diabetic	None	none	17	17	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
84	RK	ADDBH01_396	1547230	72	M	Foot	Diabetic	Purulent	Minimal	1	1	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
85	NS	ADDBH01_402	1612430	51	M	Leg	Diabetic	None	none	1	1	IPD	Gram Negative and Gram Positive	Proteus mirabilis, Escherichia coli	Incorrect
86	RS	ADDBH01_406	1582803	50	M	Perianal Region	Fournier	None	none	8	9	OPD	Gram Negative	Escherichia coli	Correct
87	MS	ADDBH01_407	1475404	80	M	Sacrum	Pressure Sore	Purulent	Minimal	1	1	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
88	BS	ADDBH01_410	1505967	78	M	Foot	Diabetic	Purulent	Minimal	1	1	IPD	Gram Negative	Klebsiella pneumoniae	Correct
89	V	ADDBH01_413	308115	33	M	Abdominal	Surgical Site Infection	Purulent	Minimal	1	1	IPD	Gram Negative	No growth	Incorrect
90	S	ADDBH01_418	1498632	62	F	Foot	Diabetic	Purulent	Minimal	2	2	OPD	Gram Negative	Escherichia coli	Correct
91	SS	ADDBH01_465	1635688	75	M	Foot	Diabetic	Purulent	Scanty	2	1	IPD	Gram Negative	Escherichia coli, Serratia marcescens	Correct
92	KK	ADDBH01_468	1634676	60	M	Sacrum	Pressure Sore	None	none	1	1	IPD	Gram Negative	Acinetobacter baumannii	Correct
93	RK	ADDBH01_477	1447454	59	M	Gluteal Region	Diabetic	Purulent	Scanty	7	7	OPD	Gram Negative	Pseudomonas aeruginosa, Escherichia coli	Correct
94	KK	ADDBH01_480	1345918	51	F	Leg	Venous ulcer	None	none	1	1	OPD	Gram Negative	No growth	Incorrect
95	RT	ADDBH01_482	1653597	29	M	Foot	Cellulitis	None	None	2	1	OPD	Gram Negative and Gram Positive	Staphylococcus Lentus, Escherichia coli	Correct
96	VK	ADDBH01_489	1655787	50	M	Foot	Diabetic	None	None	1	1	OPD	Gram Negative	Pseudomonas aeruginosa	Correct
97	LS	ADDBH01_495	566067	70	M	Foot	Diabetic	Purulent	Minimal	2	2	IPD	Gram Negative	Pseudomonas aeruginosa	Correct
98	SS	ADDBH01_501	158731	61	M	Foot	Diabetic	Purulent	Minimal	1	1	OPD	Gram Negative	Enterobacter cloacae	Correct
99	KC	ADDBH01_508	1673731	66	M	Gluteal Region	Diabetic	Purulent	Minimal	2	1	IPD	Gram Negative	Escherichia coli	Correct
100	AKB	ADDBH01_516	1679574	62	M	Foot	Diabetic	Purulent	Minimal	2	1	IPD	Gram Negative	Pseudomonas aeruginosa	Correct

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The Principal
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Ludhiana

Subject:: Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 25.09.2023 has approved the plan of thesis of Suchita, a student of your college for MS General Surgery along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
To evaluate the efficacy of a novel autofluorescence based artificial intelligence device in wound ulcers .

Supervisor
Dr. Ashvind Bawa
Assoc. Prof.,
Dept. of General Surgery

Co-Supervisor:-
Dr. Jyoti Chaudhary
Assoc. Prof.
Dept. of Microbiology

The student may submit the thesis in triplicate alongwith the plan in triplicate also, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She may be directed to attach a photocopy of this letter with the forwarding letter while submitting thesis in the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic, if any.

Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

4/69-2024

TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE BASED ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SURGERY

IN

GENERAL SURGERY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

OCTOBER, 2022

DR. SUCHITA

DEPARTMENT OF GENERAL SURGERY

DAYANAND MEDICAL COLLEGE & HOSPITAL,

LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE BASED ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS
For the degree of	Master of Surgery
Name of the candidate	Dr. Suchita
Supervisor	Dr. Ashvind Bawa, Associate Professor, Department of General Surgery, DMCH Ludhiana
Co-Supervisor	Dr. Jyoti Chaudhary, Associate Professor, Department of Microbiology, DMCH Ludhiana
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Illuminate®, a novel handheld device developed based on multi-spectral imaging of autofluorescence exhibited by bacterium has proven to be an early screening device in the identification and classification of bacteria. The non-invasive and less time-consuming procedure are the added advantages of the device. Routine microbial-based culture methods, the current standard for identifying and classifying bacteria infecting the wounds, requires taking a swab or tissue from the infected region and take 2 to 3 days for diagnosis

Aim of Study: •To rapidly identify and classify gram positive and negative organisms in wounds using artificial intelligence device.

•To evaluate predictability and see efficacy of artificial intelligence device in gram positive or gram negative infections in wounds.

Materials & Methods:

- The present study is a prospective observational study to be carried out in the department of general surgery, Dayanand medical college and hospital, Ludhiana.
- Patient's wound imaging will be taken subsequently using illuminate® and at the same time wound tissue will be sent for culture. Illuminate® suggested gram positive and negative forms will be compared to culture report from the tissue.
- If the device reports no infection, in a wound with clinical signs of infection, a tissue culture would be sent and findings will be compared with culture report.
- Sample size will be the number of patients evaluated with wound ulcers in one year duration using the artificial intelligence (AI) device.

Clinical Significance : The AI device provides rapid & accurate method of detecting infections & classifying the pathogens present in the wound in short time. This is a non-invasive point of care device and there is no need to inject a fluorescent dye or marker to observe the auto-fluorescence properties of the wound which will help in quicker detection of infection in the wound.

Keywords: Autofluorescence, Illuminate®, Gram-positive & Gram-negative bacteria, Wound, Infections

(Signature) Candidate	(Signature) Co-supervisor	(Signature) Supervisor	(Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE BASED ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS"** exist in the Department of General Surgery at Dayanand Medical College and Hospital, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis are genuine and is collected by the candidate herself under our supervision and guidance.

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APPROVAL PROFORMA BY
RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Suchita
Department	General Surgery
Topic of Thesis	CLINICAL STUDY TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE BASED ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS
Likely date of appearing in PG examination	May/ June 2024
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Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Harpreet Kaur (Professor & Head, Pathology)	Mr. R K Sharma (Legal Expert)
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Dr. Sandeep Puri
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PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

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2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA
3	COURSE OF STUDY AND SUBJECT	MS GENERAL SURGERY
4	DATE OF ADMISSION OF COURSE	7 MARCH, 2022
5	TITLE OF THE TOPIC	TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE BASED ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

In some ways, the history of wound healing is the history of humanity. A clay tablet from 2200 BC is one of the earliest medical writings known to man. This tablet may be the first to describe the "three healing gestures"— washing the wounds, producing plasters, and bandaging the wounds.[1-3] Normal wound healing is a dynamic and complicated process that consists of three phases: inflammation, proliferation, and remodelling. If the normal healing process is disrupted, an ulcer may become chronic owing to a lack of growth hormones and cytokines, which will delay the healing process.[4]

Wounds reflect a break in the skin's anatomic and physiologic continuity. Wounds may be characterised as either acute or chronic in terms of healing. A wound is deemed chronic if it does not heal within the time frame predicted by its aetiology and location. Chronic wounds are classed as either normal or unusual. Ischemic, neurotrophic, and hypostatic ulcers are common, as are two distinct entities: diabetic foot and decubitus ulcers. Eighty percent of chronic wounds on the lower leg are caused by chronic venous insufficiency, 5-10% by arterial aetiology, and the rest are predominantly neuropathic ulcers.[5]

Infections are the most prevalent cause of morbidity and death linked with foot ulcers, accounting for 40-80% of cases.[6] Exposed subcutaneous tissue provides a favourable substrate for a wide range of bacteria to contaminate and colonise, causing devitalization of the affected tissue and compromising the human immune response, making the circumstances ideal for microbial development.[7] Routine microbial-based culture methods, the current standard for identifying and classifying bacteria infecting wounds, necessitate a swab or biopsy from the infected region and take 2 to 3 days to diagnose, resulting in a delay in providing first-line treatment and/or inappropriate antibiotic use, which leads to the development of bacterial resistance.[8]

Different bacteria have varied levels of these fluorophores in different micro-environments, allowing different species of bacteria to be distinguished.[9] The interplay between the inflammatory process and

pathogens is significant to consider in the diagnosis of skin ulcer infection due to the overlap in symptoms and indications. Bacteria in ulcers often follow a progression from contamination to colonisation, from colonisation to significant colonisation and eventually infection.[10] The significance of colonisation in wound healing is unknown, however aerobic or facultative species such as Staphylococcus Aureus, Pseudomonas Aeruginosa, and beta-hemolytic Streptococci are usually blamed. The stated significance of anaerobic species may be due to the absence of anaerobic culture techniques in studies rather than a lack of virulence. Anaerobic organisms are more difficult to grow and identify, yet they account for 30% of total microbial isolates in wounds.[11-13]

The nature of bacterial colonisation varies throughout time. In wounds that are less than one month old, cutaneous and Gram-positive organisms predominate. As ulcer healing progresses, a greater range of organisms, including Gram-negative organisms such as coliforms, anaerobes, and Pseudomonas species, may colonise the wound.[14]

Illuminate®, an unique portable device based on multispectral imaging of bacterial autofluorescence, has shown to be an early screening device in the identification and categorization of bacteria. The gadget examines both metabolic and infectious indicators of the underlying pathogen, showing high overall accuracy but failing to identify commensals. The device's other benefits include a non-invasive and less time-consuming treatment.[15] The usage of an artificial intelligence device (Illuminate) for infection diagnostics should be seen as an innovative method. Swabs may give useful information about wounds that do not heal or exhibit signs of serious colonisation. Culture may discover resistant organisms and focus systemic therapy for infected wounds by indicating the prevalent flora in such wounds.

Hence, in this study to take place in DMCH Ludhiana, we will use the Artificial Intelligence device to identify the types of microbes in the wound ulcers. In addition, the results of this study may help in initiating an early antibiotic therapy and further wound management.

6.2 REVIEW OF LITERATURE

The traditional definition of wounds and ulcers was primarily based on aetiology, with a wound (now called an acute or surgical wound) being said to be caused by violence (eg, an outside force such as a bullet, a surgical incision) and an ulcer, now called a chronic "wound," being caused by some kind of internal aetiology (eg, venous hypertension with its secondary consequences to skin integrity).[16]

CLASSIFICATION OF ULCERS

PATHOLOGICALLY, the ulcers can be classified into:

(A) Non-specific ulcers- These ulcers are further categorised into the following groups: (1) Traumatic — may be mechanical, such as a dental ulcer of the tongue caused by a jagged tooth or the pressure of a splint, or physical, such as an electrical or X-ray burn, or chemical, such as the application of caustics. (2) Arterial — as in atherosclerosis, Buerger's disease, Raynaud's disease (primary and secondary), and other conditions. (3) Venous — for example, a varicose ulcer in a postphlebitic leg. (4) Neurogenic (Trophic) — examples include bed sores and perforating ulcers. (5) Related to malnutrition, for example, tropical ulcers, which develop in the legs and feet of the persons living in tropical climates.

(B) Specific ulcers — e.g. Tuberculous, Syphilitic, Soft Sores, Actinomycosis and Meleney's ulcers.

(C) Malignant ulcers — e.g. Epithelioma, Marjolin's ulcer, Rodent ulcer and Malignant Melanoma.[17]

Grading

Wagner's Grading of Ulcer follows:[18]

Grade 0 - Pre-ulcerative lesion or healed ulcer

Grade 1 - Superficial ulcer

Grade 2 - Ulcer deeper to subcutaneos tissue exposing soft tissue or bone

Grade 3 - Abscess formation underneath, Osteomyelitis

Grade 4 - Gangrene of part of tissues, limb or foot

Grade 5 - Gangrene of entire one area or foot

Wound Ulcer Infections:

Bacteria in ulcers often follow a progression from contamination to colonisation, then to critical colonisation, and ultimately to infection.[10] The significance of colonisation in wound healing is unknown, however Aerobic or Facultative species such as Staphylococcus Aureus, Pseudomonas Aeruginosa, and beta-hemolytic Streptococci are usually blamed. The stated significance of Anaerobic species may be due to the absence of anaerobic culture techniques in studies rather than a lack of virulence. Anaerobic organisms are more difficult to grow and identify, yet they account for 30% of total microbial isolates in wounds.[11-13] The nature of bacterial colonisation varies throughout time. In wounds that are less than one month old, Cutaneous and Gram-positive organisms predominate. As the ulcer healing progresses, a greater range of organisms, including Gram-negative such as Coliforms, Anaerobes and Pseudomonas species, may colonise the wound.[14]

Techniques for diagnosing Wound infection: Culture may discover resistant organisms and focus systemic therapy for infected wounds by indicating the prevalent flora in such wounds. Routine microbial-based culture method is the current gold standard for identifying and classifying the bacteria infected wounds, which necessitate a swab or biopsy from the infected region and takes 2 to 3 days to diagnose, resulting in a delay in providing first-line treatment and/or inappropriate antibiotic use, which leads to the development of bacterial resistance.[8] Illuminate®, a revolutionary hand device based on multi-spectral imaging of bacterial autofluorescence, has shown to be an early screening device in the identification and categorization of bacteria. The gadget examines both metabolic and infectious indicators of the underlying pathogen, showing high overall accuracy but failing to identify commensals. The device's other benefits include a non-invasive and less time-consuming treatment.[15] The usage of an artificial intelligence device (illuminate) for infection diagnostics should be seen as an innovative method.

Clinical studies based on the artificial intelligence device:

1. A clinical study to evaluate autofluorescence imaging of diabetic foot ulcers using a novel artificial intelligence enabled noninvasive device based on the artificial intelligence device Viswanathan V, Govindan S, Selvaraj B, Rupert S, and Kumar R conducted the study, published in The international journal of lower extremity wounds was completed in October 2021. The purpose of this research was to compare the device's diagnostic accuracy in identifying bacterial gram type to established culture procedures. A total of 178 diabetic patients were recruited from a tertiary care centre, and the surgeon took 203 tissue samples from the wound base.

The investigator used the equipment to acquire wound pictures. Tissue samples were collected from the color-coded diseased zone identified by the device's artificial intelligence system and submitted for microbiological analysis. The gadget was shown to have an accuracy of 89.54%, a positive predictive value of 86.27% for identifying Gram-positive bacteria, 80.77% for gram-negative bacteria and 91.67% for no infection when the findings were compared to the gram type inferred by the device. The negative predictive value was 87.25% for gram-positive bacteria, 92% for gram-negative bacteria and 96.12% for no infection. The findings demonstrated the accuracy of this innovative autofluorescence device in recognising and categorising gram-positive bacteria, as well as its potential to considerably assist doctors in early infection evaluation and treatment.

2. Parekh, J.N., Soni, P., Meena, M.K., et al. conducted a clinical research titled " A Novel Device and Technology for Detecting Bacterial Infection and Gram type in Diabetic Foot Ulcers" according to Indian J Surg (2022), which concluded that among the various recently developed methods for detecting bacterial illness, procedures based on bacteria's fluorescence may be simple, cost-effective, and quick.

The microbiology section provided a culture report for 149 tissue samples, which was compared to the Gram type of infection indicated by the device. Gram-negative bacteria were shown in green overlay on the device imaging report, whereas Gram-positive bacteria were shown in red overlay. If two varieties of Gram-negative bacteria were present

in the wound, the gadget just displayed them as Gram-negative bacteria since it does not distinguish between distinct types of bacteria species. This is also true for Gram-positive bacteria. In the instance of a polymicrobial illness including both Gram-positive and Gram-negative bacteria, the gadget indicated the Gram type of the bacteria that are more common in the location.

The new gadget's sensitivity was found to be 99.24%, which implies that the new equipment would correctly diagnose bacterial wound infection 99.24% of the time.

The device's specificity was 82.35%, allowing it to properly identify those samples that are free of illness.

Cohen's Kappa (K) was also used to assess the degree of agreement between the culture technique and the new device approach. K = 0.86 (P 0.001) was found , indicating that there was extremely substantial corelation between the culture technique and the novel device approach. As a result, the new technology accurately identified bacterial infections. There is a strong link between the culture technique and the novel gadget approach for identifying illness and the Gram type of bacteria. Cramer's V test was used to determine the strength of relationship between culture technique and new device method. This screening equipment provides a number of benefits.

(1) The presence of infection in the ulcer as well as the Gram type of the infecting bacteria may be determined promptly. Using the hospital antibiogram, suitable antibiotics depending on Gram type of bacteria may be begun.

(2) The device can measure the size of the wound automatically, assisting the doctor during follow-up. The treating doctor can quickly determine the state of the infection as well as any decrease in the size of the ulcer.

(3) It can detect bacterial burdens as low as 10^3 CFU/gm (Colony forming unit per gramme), allowing for early action.

(4) In this study, when tissues sample were taken under the guidance of device, the report showing "no growth" were significantly less. "No growth" was reported in 28 patients when tissues were taken blindly

without using the device and only 15 patients when tissues were taken under guidance of the device. This difference is statistically significant. Overall accuracy of the device is 93.20% in diagnosing Gram type of infection.

(5) The device is beneficial in OT for receiving quick input during debridement of an infected wound.

(6) Prior to grafting the partial thickness skin graft on the recipient region, the device may scan the area and reveal any infection pockets, reducing graft rejection.

The device has certain drawbacks.

(1) Imaging the wound requires a dark environment or black hoods. In the presence of ambient light, the wound cannot be visualized.

(2) At the moment, the device cannot distinguish the species of bacteria, although traditional culture methods can tell us the species of bacteria, however the device can only differentiate infecting bacteria as Gram-negative or Gram-positive.

(3) When there is a polymicrobial illness, the device cannot distinguish between different species of bacteria and will only indicate the Gram type of the most prevalent bacterium. It becomes difficult to determine the actual number of polymicrobial infections in a given site.

3. In 2020, Geethanjali Radhakrishnan, Aayush Gupta, John King, and Devina Ganvir conducted a research on Rapid handheld screening device to identify skin and soft tissue infections, where they demonstrated A portable device (Illuminate) that used the natural autofluorescence features of bacterial and fungal species to non-invasively and swiftly detect infections on SSTI utilizing multispectral photography, image processing, and machine learning algorithms. Components of Device were:

Fluorescence imaging module, image acquisition module, image processing module, display module, and control system

A. Fluorescence Imaging Module: The fluorescence imaging module is made up of multi-wavelength UV LEDs (UV-A, UV-B, and visible) operated by an LED driver board, an optical filter wheel with emission filters over a CMOS imaging sensor, and a CMOS imaging sensor. A

tiny servo motor turns the filter wheel. The whole module is managed by a control system that sequences excitation/emission wavelengths and takes a multispectral picture of the wound.

B. Control System: The control system is comprised of a four core ARMv8 Cluster running BCM2837 software. The control system gets instructions from the Android Smart Phone's Display Module. It is in charge of the fluorescent imaging module, as well as the image acquisition, processing, and display modules.

C. Image Acquisition Module: The Android smartphone's 13MP CMOS sensor is used for image acquisition. The control system automatically sets the frame rate and exposure for photos taken prior to image collection.

D. Display module: A smartphone application that allows the user to input patient information, configure the SSTI area on the capture window, collect photographs, mark the wound region after capture, and ultimately display a colour code wound map with specifics of the infected region. Red represents gram positive bacteria, green represents gram negative bacteria, and blue represents fungus. The interface also allows doctors to share and print reports, access patient data at any time, and make notes.

E. Image Processing Module: The image processing module analyses the autofluorescence response acquired from the spectrum photographs, as well as the different biomolecular fluorescent intensities given by NADH, Flavin, Pyoverdine, Chitin, Porphyrin, and any unknown molecule. After capturing the spectrum pictures, the image processor employs an image orientation mapper to adjust for orientation mismatches. Each picture is aligned and orientated, and the mean intensity in the area of interest for each filter is determined and utilized for machine learning.

F. Pathogen classifier model: Spectral pictures from the illumination device are acquired and transferred to a decision matrix stage, where the images are filtered based on hue and threshold to separate autofluorescence areas from noise and other undesired signals. The filtered portions are referred to as ROIs (regions of interest), and they

are delivered as a swab or tissue to a microbiology lab, where a report with the organism name used for labelling is generated. The matching ROI spectrum is given into a Machine learning system, which predicts and shows the pathogen gram type. In the instance of fungus, a basic thresholding is performed and the data is passed to ML.

It was a single-arm interventional research in which the device findings were compared to a gold standard culture technique for bacteria or to KOH staining for fungus.

A swab/deep tissue or skin scraping was submitted for microbiology analysis in the area where the gadget detected the presence of bacteria/fungi. The microbiological report was compared to the device report. The research covered patients with fungal infections, surgical site infections, contact dermatitis, stasis ulcers (both arterial and venous), diabetic foot ulcers, sterile ulcers, any scars/suture site, sinuses/fistulas. Patients who were unable to consent were ruled out of the trial.

182 patients gave informed consent and were scanned using the gadget. The gadget was used to photograph 33 patients with different types of SSTI and 149 dermatology-related fungal infections.

The study's findings with >85% accuracy illustrate the device's appealing potential in any SSTI context. Because the findings of the gram type categorization are rapid, the device greatly aids the Clinicians in providing appropriate first-line antibiotic therapy and minimising antibiotic overuse.

6.3 AIMS AND OBJECTIVES OF THE STUDY

•To rapidly identify and classify gram positive and negative organisms in wounds using artificial intelligence device.

•To evaluate predictability and see efficacy of artificial intelligence device in gram positive or gram negative infections in wounds.

7. MATERIALS AND METHODS

- The present study is a prospective observational study to be carried out in the department of general surgery, Dayanand Medical College and Hospital, Ludhiana.
- Patient's wound imaging will be taken subsequently using Illuminate® and at the same time wound tissue will be sent for culture. Illuminate® suggested gram positive and negative forms will be compared to culture report from the tissue.
- If the device reports no infection, in a wound with clinical signs of infection, a tissue culture would be sent and findings will be compared with culture report.
- Sample size will be the number of patients evaluated with wound ulcers in one year duration using the device.

7.1 Source of Data:

Data shall be collected from all the patients with wound ulcers who are admitted or came during the given time period will be taken for study considering the inclusion and exclusion criteria

7.2 METHOD OF COLLECTION OF DATA

Inclusion Criterion

1. All wounds, ulcers of varying etiology like diabetic ulcers, venous ulcers, arterial ulcers, post-op wounds and traumatic ulcers will be taken.
2. Both acute and chronic ulcers will be taken.
3. Both inpatients and outpatients will be taken.

Exclusion Criterion

1. Age less than 18 years.
2. Underlying bony involvement like osteomyelitis.
3. Patients with existing skin disease like eczema, psoriasis in the area close to the wounds.
4. Patients not giving consent for the study.

Duration of Study/ Number of Cases Sample size will be the number of patients evaluated with wound ulcers in one year duration using the artificial intelligence (AI) device.

Outcome: Knowing the efficacy of the artificial intelligence device in differentiating gram positive or negative infection or no infection in wound ulcer

Statistical Analysis (Name of the method in plan): Data will be described in terms of range; mean ±standard deviation (± SD), frequencies (number of cases) and relative frequencies (percentages) as appropriate. For comparing categorical data, Chi square ($\chi 2$) test will be performed and fisher exact test will be used when the expected frequency is less than 5. Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value will be calculated. A probability value (*p* value) less than 0.05 will be considered statistically significant. All statistical calculations will be done using (Statistical Package for the Social Science) SPSS 21 version (SPSS Inc., Chicago, IL, USA) statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

1. Wound tissue cultures
2. Photograph capture in the Artificial intelligence device.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes, Attached herewith

8. LIST OF REFERENCES

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PROFORMA

Sr. No._____

Name - Age - Sex -

CR. No. - Date - Illuminate ID _____

Presenting Complaint -

Wound Etiology -

- Traumatic
- Diabetic
- Post-Op
- Arterial
- Venous
- Autoimmune
- Others (specify) -

GPE -

BP - Pulse -

Respiratory rate - Temperature -

Presence of risk factor -

- BMI >30
- Diabetes Mellitus
- CKD
- Immunosuppression
- Alcoholism
- CLD
- Malignancy
- Burns
- TB
- H/O Surgery
- H/O Childbirth
- BMI <18
- Cardiac Diseases
- Others -

Signature of candidate Signature of supervisor

Local Examination -

Site -

Extent -

Exudate Type -

Wound Size -

Investigations -

Illuminate imaging result -

Wound tissue culture result -

Comparison of both results -

Signature of candidate

Signature of supervisor

INFORMED PATIENT CONSENT

Date: _____

I…….D/o, W/o, S/o……..…… am willing to participate in the study **"TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

……. ……
Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____	_____
गवाह के हस्ताक्षर	रोगी के हस्ताक्षर/अंगूठे का निशान
नाम:	नाम:

_____

अन्वेषक के हस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

“TO EVALUATE THE EFFICACY OF A NOVEL AUTOFLUORESCENCE ARTIFICIAL INTELLIGENCE DEVICE IN WOUND ULCERS” ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ-ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾ ਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ – ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ।

ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

CONSENT FORM FOR IMAGE CAPTURING

Date:

I understand that treating doctor will capture images of my wound with an AI based device.

I understand that this device will also use fluorescence to identify the presence and type of infection in the wound.

I understand that my images will be used for research articles and publications in journal.

I hereby give my informed consent for all the above statements and wish to enroll in this study.

…….

Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**